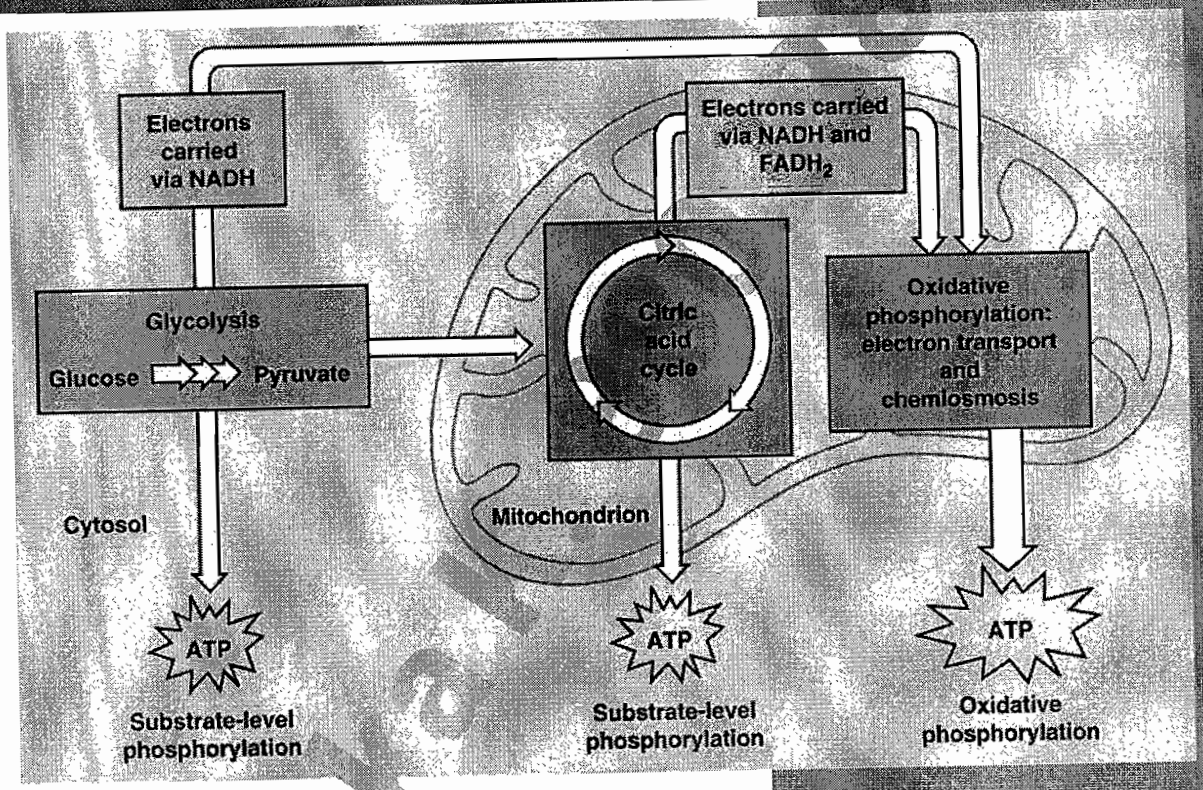
evolution

2017

BOTANY: Plant Biochemistry



evolution

Reference Study Material

Phone: +91 11 47092329; +91 11 32974645 | Web: www.evolution.co.in | Mail: info@evolution.co.in

Plant Biochemistry

Table of contents

Please refer to the PLANT PHYSIOLOGY Booklet for coverage of the following biochemistry topics:

1. **Enzymes**
2. **Plant Hormones**
3. **Nitrogen metabolism**

Part – I: Respiration

Table of contents

AN OVERVIEW OF RESPIRATION

Respiration is a term with dual meaning in biology:

1. The physical process by which organisms take in oxygen from the surrounding medium and emit carbon dioxide. In this sense the process is also known as physical respiration, breathing, ventilation or gas exchange

2. The chemical process by which fuel molecules such as sugars and fats are broken down within a cell to liberate energy for cellular life processes. In this sense it is also known as chemical, biochemical, or cellular respiration.

Cellular respiration has two versions.

1. **Aerobic respiration**, which is a more efficient mode of respiration because it liberates more energy from the food molecule, occurs in the presence of molecular O_2. The Krebs cycle or citric acid cycle, followed by the Oxidative Phosphorylation steps form the core of aerobic respiration. Nearly all the eukaryotes and most of the prokaryotes today carry out aerobic respiration.

2. **Anaerobic respiration** is far less efficient than the aerobic version but it can take place even in the absence of molecular oxygen. This process incorporates the chemical pathway of glycolysis and results in lactic acid or ethyl alcohol. It occurs in certain some bacteria, archaea and certain eukaryotic microbes such as Trichomonads and some fungi. It is also used by higher eukaryotes as a reserve or emergency form of energy liberating process when oxygen is in short supply.

A BIOCHEMICAL LOOK ON AEROBIC RESPIRATION

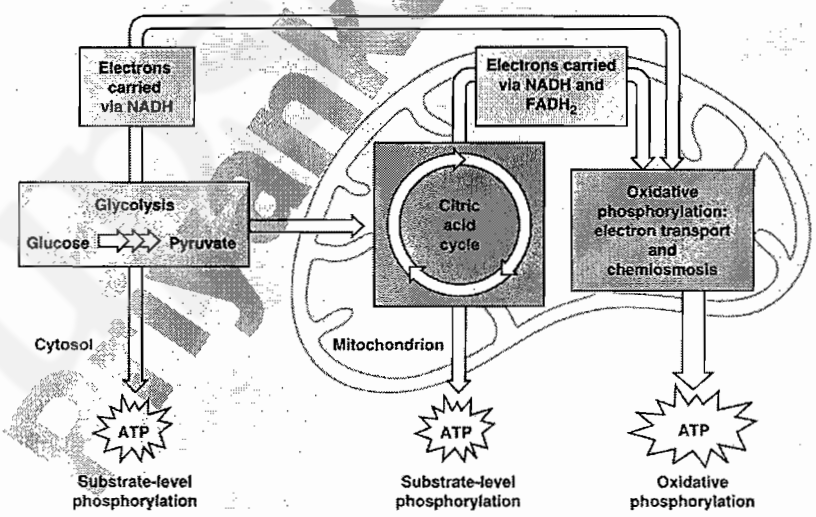


FIGURE 1: An overview of respiration

Aerobic respiration is essentially a series of enzyme catalyzed steps in which the C-C bonds of food molecules are broken by oxidation within the cells, leading to release of considerable amount of bond energy that is ultimately conserved in ATP molecules. During this oxidative process electrons are sequentially extracted from the food molecule and ultimately transferred to O_2, leading

to the formation of H_2O. The transfer of electrons from food molecules to O_2 is carried out by two types of electron carriers: NAD^+ and FAD. The carbon skeleton intermediates produced during respiration can be used as precursors for biosynthesis of other molecules in the cell.

The reaction, assuming Glucose as the food substrate, can be represented by the general chemical equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \sim 30\ ATP$$

The compounds that are oxidized during the process are known as *respiratory substrates*. Usually carbohydrates are oxidised to release energy, but proteins, fats and even organic acids can be used as respiratory substances, under certain physiological conditions.

The ATP molecules generated by respiration act as the *energy currency of the cell*. It means that ATP is broken down whenever and wherever energy needs to be utilized within a cell. A large amount of free energy is liberated when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (P_i) or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PP_i).

$$ATP + H_2O \rightleftharpoons ADP + P_i \qquad \Delta G^{\circ\prime} = -7.3\ kcal\ mol^{-1}\ (-30.5\ kJ\ mol^{-1})$$

$$ATP + H_2O \rightleftharpoons AMP + PP_i \qquad \Delta G^{\circ\prime} = -10.9\ kcal\ mol^{-1}\ (-45.6\ kJ\ mol^{-1})$$

A BIOENERGETIC LOOK ON AEROBIC RESPIRATION

Aerobic respiration can also be described as a series of enzyme catalyzed energy transformation reactions occurring in the presence of O_2 during which the chemical bond energy trapped in highly reduced food molecules is sequentially transformed into the phosphoryl group transfer potential of ATP. The major energy transformation landmarks in aerobic respiration are described below.

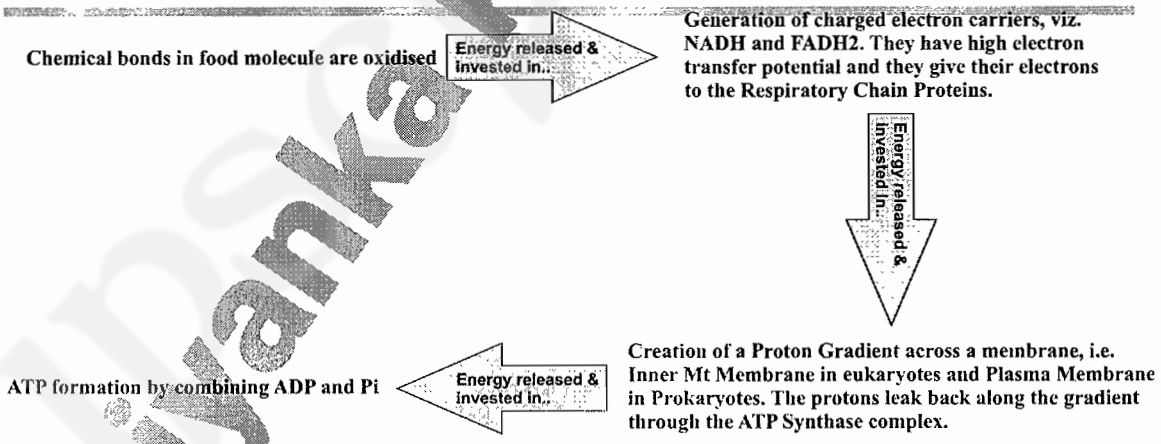
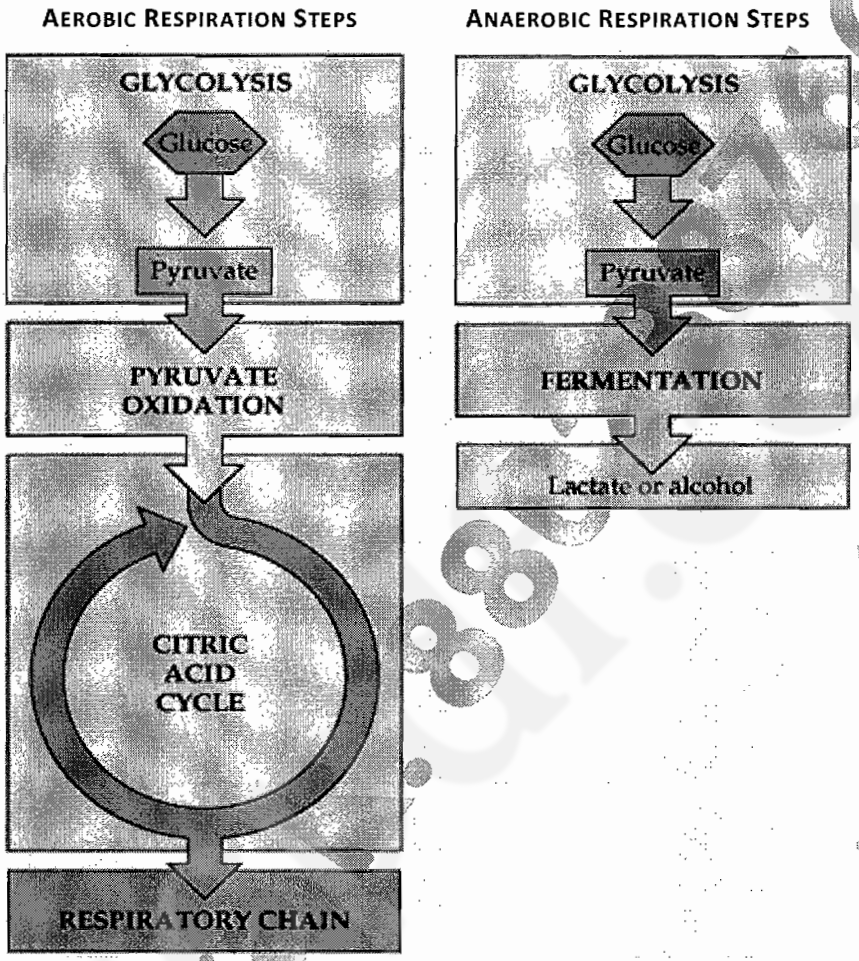


FIGURE 2: The bioenergetic transformations in respiration

BROAD STEPS OF RESPIRATION



GLYCOLYSIS

INTRODUCTION TO ROLES OF GLUCOSE AND GLYCOLYSIS

The many roles of Glucose

Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms.

1. It is relatively rich in potential energy, and thus a good fuel; the complete oxidation of glucose to carbon dioxide and water proceeds with a standard free-energy change of 2,840 kJ/mol.

2. It is also a versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions. A bacterium such as *Escherichia coli* can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid, or other metabolic intermediate it needs for growth.

3. Glucose is also used in synthesizing a high molecular weight polymer such as starch or glycogen, with which a cell can store significant amounts of free energy in large quantities of hexose units while maintaining a relatively low cytosolic osmolarity. When energy demands increase, glucose can be released from these intracellular storage polymers and used to produce ATP either aerobically or anaerobically.

In animals and vascular plants, glucose has three major fates:

1. it may be stored (as a polysaccharide or as sucrose);

2. oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates; or

3. oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes.

Glycolysis

Glycolysis is an almost universal central pathway of glucose catabolism, the pathway with the largest flux of carbon in most cells. In glycolysis (from the Greek *glykys*, meaning "sweet," and *lysis*, meaning "splitting"), a molecule of glucose is degraded in a series of ten enzyme-catalyzed reactions to yield two molecules of the three-carbon compound pyruvate. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH.

Thus, in the sequential reactions of glycolysis, three types of chemical transformations are particularly noteworthy:

1. Degradation of the carbon skeleton of glucose to yield pyruvate,

2. Phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis, and

3. Transfer of a hydride ion to NAD, forming NADH.

The glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, and sperm, for example). Some plant tissues that are modified to store starch (such as potato tubers) and some aquatic plants (watercress, for example) derive most of their energy from glycolysis; many anaerobic microorganisms are entirely dependent on glycolysis.

Glycolysis was the first metabolic pathway to be elucidated and is probably the best understood. The important landmarks in the studies of glycolysis have been:

1. Eduard Buchner's discovery in 1897 of fermentation in broken extracts of yeast cells,

2. The elucidation of the whole pathway in yeast by Otto Warburg and Hans von Euler-Chelpin in 1932; and

3. The elucidation of the pathway in muscle cells by Gustav Embden and Otto Meyerhof in 1932.

THE STEPS OF GLYCOLYSIS

The reactions

As displayed in Figure 1, the breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps, the first five of which constitute the preparatory phase.

Step 1. In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield glucose 6-phosphate, with ATP as the phosphoryl donor. This reaction, which is irreversible under intracellular conditions, is catalyzed by *hexokinase*.

Step 2. The D-glucose 6-phosphate thus formed is converted to D-fructose 6-phosphate by the enzyme *phosphoglucose isomerase*. From a chemical perspective, the isomerization in step 2 is critical for setting up the phosphorylation and C-O-C bond cleavage reactions in steps 3 and 4, respectively.

Step 3. D-fructose 6-phosphate is again phosphorylated, this time at C-1 by the catalytic action of *phosphofructokinase -1 (PFK-1)*, to yield D-fructose 1,6-bisphosphate. For this phosphorylation too, ATP is the phosphoryl group donor. This enzyme is called PFK-1 to distinguish it from a second enzyme (PFK-2) that catalyzes the formation of fructose 2,6-bisphosphate from fructose 6-phosphate in a separate pathway. The PFK-1 reaction is essentially irreversible under cellular conditions, and it is the first *"committed" step* in the glycolytic pathway; glucose 6-phosphate and fructose 6-phosphate have other possible fates, but fructose 1,6-bisphosphate is targeted for glycolysis.

Some bacteria and protists and perhaps all plants have a phosphofructokinase that uses pyrophosphate (PPi), not ATP, as the phosphoryl group donor in the synthesis of fructose 1,6-bisphosphate.

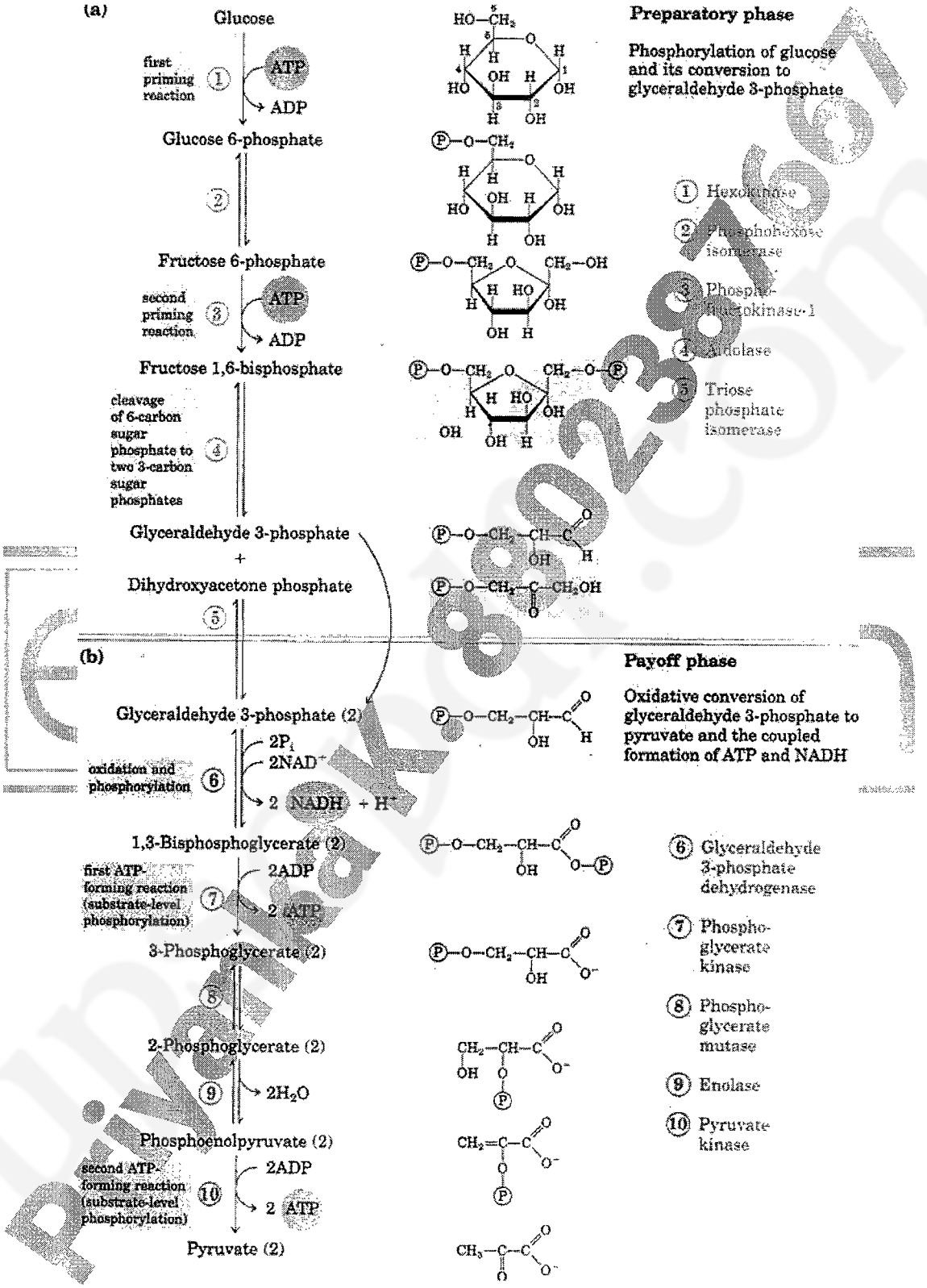


FIGURE 1: The steps of glycolysis

Step 4. Fructose 1,6-bisphosphate is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. This is the "lysis" step that gives the pathway its name. The enzyme for this step is *fructose 1,6-bisphosphate aldolase*, often called simply *aldolase*, which catalyzes a reversible aldol condensation. There are two classes of aldolases. Class I aldolases, found in animals and plants, use the mechanism involving a Schiff base. Class II enzymes, in fungi and bacteria, do not form the Schiff base intermediate.

Step 5. Only one of the two triose phosphates formed by aldolase, glyceraldehyde 3-phosphate, can be directly degraded in the subsequent steps of glycolysis. The other product, dihydroxyacetone phosphate, is rapidly and reversibly isomerized to a second molecule of glyceraldehyde 3-phosphate by the enzyme, *triose phosphate isomerase*: thus ending the first phase of glycolysis.

To summarizethe first or the preparatory phase of glycolysis, we can say that in this phase of glycolysis the energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted into a common product, glyceraldehyde 3-phosphate.

Step 6. Each molecule of glyceraldehyde 3-phosphate is oxidized and phosphorylated by inorganic phosphate (not by ATP) to form 1,3-bisphosphoglycerate by the enzyme *glyceraldehyde 3-phosphate dehydrogenase*. The acceptor of hydrogen in the glyceraldehyde 3-phosphate dehydrogenase reaction is NAD. The reduction of NAD produces NADH.

Step 7. Energy is released when the enzyme *phosphoglycerate kinase* transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate. This is a substrate-level phosphorylation reaction, where an ATP molecule is generated.

Step 8. Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate occurs when the enzyme *phosphoglycerate mutase* catalyzes a reversible shift of the phosphoryl group between C-2 and C-3 of glycerate.

Step 9. Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate occurs in the second glycolytic reaction that generates a compound with high phosphoryl group transfer potential. The enzyme catalyzing this step is *enolase* that promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield phosphoenolpyruvate (PEP).

Step 10. The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by *pyruvate kinase*, which requires K^+ and either Mg^{2+} or Mn^{2+}. The end product of this step is pyruvate. In this substrate-level phosphorylation reaction, an ATP molecule is generated.

In the overall glycolytic process, one molecule of glucose is converted to two molecules of pyruvate (*the pathway of carbon*). Two molecules of ADP and two of Pi are converted to two molecules of ATP (*the pathway of phosphoryl groups*). Four electrons, as two hydride ions, are transferred from two molecules of glyceraldehyde 3-phosphate to two of NAD^+ (*the pathway of electrons*).

Fates of pyruvate

With the exception of some interesting variations in the microbial realm, the pyruvate formed by glycolysis is further metabolized via one of three catabolic routes (Figure 2).

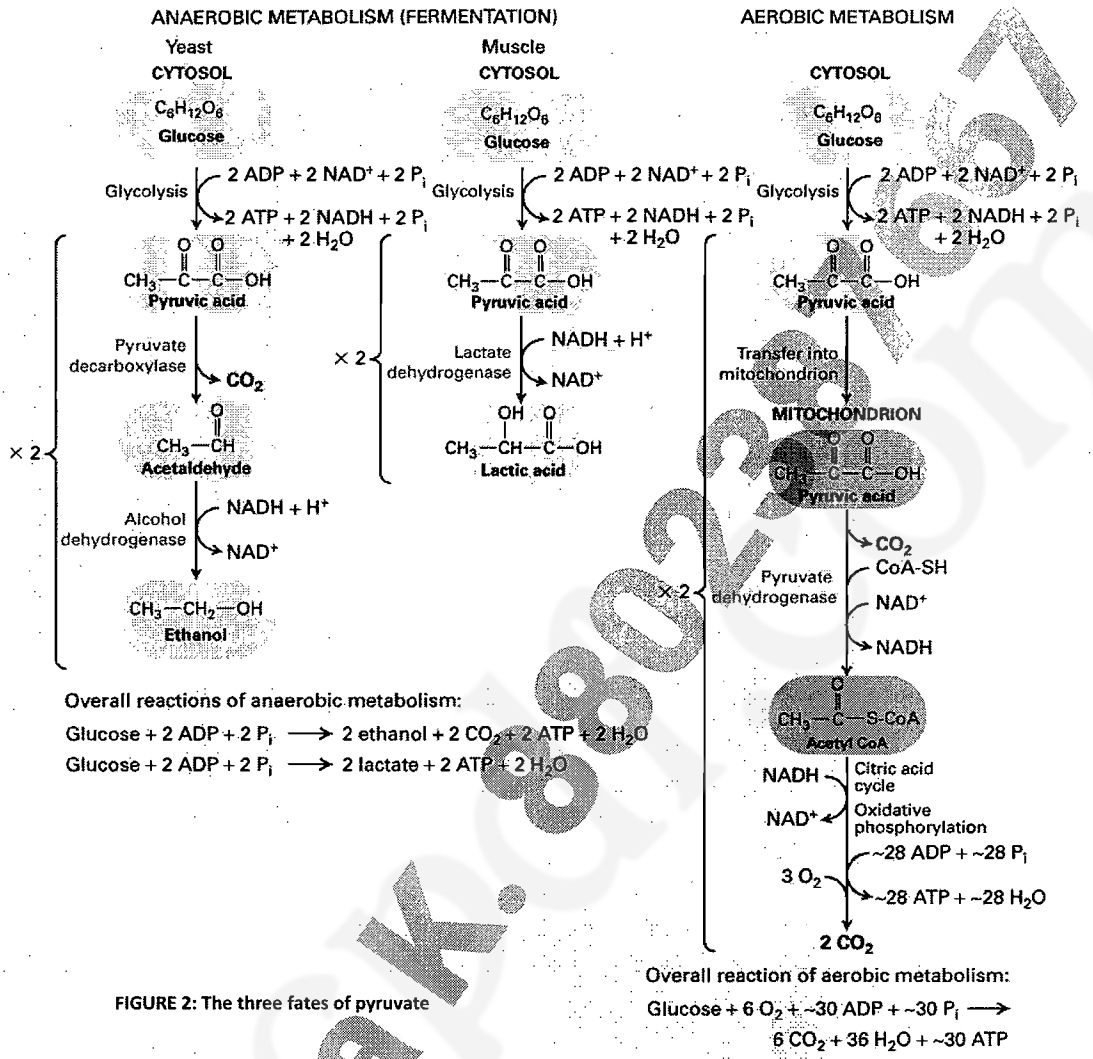


FIGURE 2: The three fates of pyruvate

1. Glycolysis releases only a small fraction of the total available energy of the glucose molecule; the two molecules of pyruvate formed by glycolysis still contain most of the chemical potential energy of glucose, energy that can be extracted by oxidative reactions in the citric acid cycle and oxidative phosphorylation. In aerobic organisms or tissues, under aerobic conditions, glycolysis is only the first stage in the complete degradation of glucose. Pyruvate is oxidized, with loss of its carboxyl group as CO_2, to yield the acetyl group of acetyl-coenzyme A; the acetyl group is then oxidized completely to CO_2 by the citric acid cycle. The electrons from these oxidations are passed to O_2 through a chain of carriers in the mitochondrion, to form H_2O. The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion.

2. The second route for pyruvate is its reduction to lactate via lactic acid fermentation. When vigorously contracting skeletal muscle must function under low-oxygen conditions (hypoxia), NADH cannot be reoxidized to NAD, but NAD is required as an electron acceptor for the further oxidation of pyruvate. Under these conditions pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD necessary for glycolysis to continue. Certain tissues and cell types (retina and erythrocytes, for example) convert glucose to lactate even under aerobic conditions, and lactate is also the product of glycolysis under anaerobic conditions in some microorganisms.

3. The third major route of pyruvate catabolism leads to ethanol. In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO_2, a process called ethanol (alcohol) fermentation.

The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can, for example, provide the carbon skeleton for the synthesis of the amino acid alanine.

REGULATION OF GLYCOLYSIS

Flux through the Glycolysis pathway is regulated by control of the 3 enzymes that catalyze highly spontaneous reactions:

1. Hexokinase,
2. Phosphofructokinase, &
3. Pyruvate Kinase.

1. Hexokinase, the first step in the Glycolysis pathway, is inhibited by its product glucose-6-phosphate:
   a. by competition at the active site, and
   b. by allosteric interaction at a separate site on the enzyme.

   Cells trap glucose by phosphorylating it, preventing exit on glucose carriers. Product inhibition of Hexokinase ensures that cells will not continue to accumulate glucose from the blood, if glucose-6-phosphate within the cell is ample.

   Glucokinase is a variant of Hexokinase found in liver. Glucokinase has a high K_M for glucose. It is thus active only at high glucose. One effect of insulin, a hormone produced in response to high blood glucose, is activation in liver of transcription of the gene that encodes the Glucokinase enzyme. Glucokinase is not subject to product inhibition by glucose-6-phosphate. The liver will take up and phosphorylate glucose even when liver glucose-6-phosphate is high. Glucokinase, with its high KM for glucose, allows the liver to store glucose as glycogen in the fed state when blood [glucose] is high. The liver enzyme Glucose-6-phosphatase catalyzes hydrolytic release of Pi from glucose-6-phosphate. Thus glucose is released from the liver to the blood as needed to maintain blood glucose.

   Liver Glucokinase is subject to inhibition by a glucokinase regulatory protein (GKRP). The ratio of Glucokinase to GKRP in liver changes in different metabolic states, providing a mechanism for modulating rates of glucose phosphorylation.

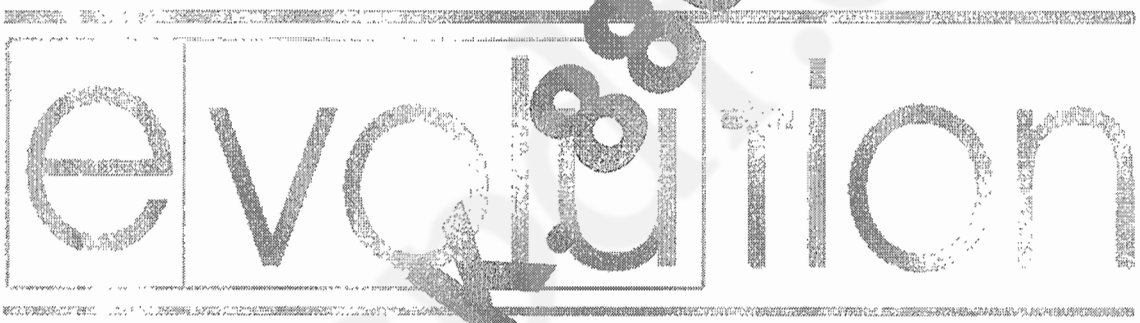
2. Pyruvate Kinase, the last step of the Glycolysis pathway, is controlled in liver partly by modulation of the amount of enzyme. High glucose within liver cells causes a transcription factor carbohydrate responsive element binding protein (ChREBP) to be transferred into the nucleus, where it activates transcription of the gene for Pyruvate Kinase. This facilitates converting some of the excess glucose to pyruvate, which is metabolized to acetyl-CoA, the main precursor for synthesis of fatty acids, for long term energy storage.

3. Phosphofructokinase is usually the rate-limiting step of the Glycolysis pathway. Phosphofructokinase catalyzes:

   fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP

   a. Phosphofructokinase (PFK) is allosterically inhibited by ATP. At low concentration, the substrate ATP binds only at the active site. At high concentration, ATP binds also at a lower-affinity regulatory site, promoting the tense conformation. The tense conformation of Phosphofructokinase has a lower affinity for its other substrate, fructose-6-phosphate. Inhibition of Phosphofructokinase, the rate-limiting step of Glycolysis, when ATP is high, prevents breakdown of glucose, in a pathway whose main role is to make ATP. It is more useful to the cell to store glucose as glycogen when ATP is plentiful.

   b. AMP, which is present at significant levels only when there is extensive ATP hydrolysis, antagonizes the effect of high ATP on PFK.



FATES OF PYRUVATE UNDER ANAEROBIC CONDITIONS: FERMENTATION

INTRODUCTION TO FERMENTATION

As discussed earlier, the pyruvate formed by glycolysis is further metabolized via one of three catabolic routes (Figure 2 on page 5).

Under aerobic conditions pyruvate is oxidized to acetate, which enters the citric acid cycle and is oxidized to CO_2 and H_2O, and NADH formed by the dehydrogenation of glyceraldehyde 3-phosphate is ultimately reoxidized to NAD^+ by passage of its electrons to O_2 in mitochondrial respiration.

However, under hypoxic conditions, as in very active skeletal muscle, in submerged plant tissues, or in lactic acid bacteria, NADH generated by glycolysis cannot be re-oxidized by O_2. Failure to regenerate NAD^+ would leave the cell with no electron acceptor for the oxidation of glyceraldehyde 3-phosphate, and the energy-yielding reactions of glycolysis would stop. NAD^+ must therefore be regenerated in some other way.

Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound. This is in contrast to cellular respiration, where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. However, Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption.

Fermentation is thus the general term for such processes, which extract energy (as ATP) but do not consume oxygen or change the concentrations of NAD^+ or NADH. Fermentations are carried out by a wide range of organisms, many of which occupy anaerobic niches, and they yield a variety of products, some of which find commercial uses.

Under anaerobic conditions, the absence of oxygen, pyruvic acid can be routed by the organism into one of two fermentative pathways:

1. lactic acid fermentation
2. alcohol fermentation

LACTIC ACID FERMENTATION

When animal tissues cannot be supplied with sufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced in glycolysis, NAD^+ is regenerated from NADH by the reduction of pyruvate to lactate. Some tissues and cell types (such as erythrocytes, which have no mitochondria and thus cannot oxidize pyruvate to CO_2) produce lactate from glucose even under aerobic conditions. The reduction of pyruvate is catalyzed by lactate dehydrogenase.

The reduction of pyruvate is catalyzed by lactate dehydrogenase uses NADH as the source of electron. Thus, the conversion of pyruvate to lactate regenerates NAD^+, which allows glycolysis to continue.

***Fate of lactate*:** Lactate diffuses out of the cell and into the blood. The lactate in the bloodstream is converted back into pyruvate in the liver, for use when oxygen is once again present. Certain cells, such as cardiac muscle cells, are highly permeable to lactate. Lactate is converted into pyruvate and metabolised normally (ie: via the Krebs Cycle). Since these cells are highly oxygenated, it is unlikely that lactate would accumulate (as is the case in oxygen-starved muscle cells). This also allows circulating glucose to be available to muscle cells.

Any excess lactate is taken up by the liver, converted to pyruvate and then to glucose. This, along with the production of lactate from glucose in muscle cells constitutes the Cori cycle.

***Regulation of lactate formation*:** Phosphofructokinase (PFK) is inhibited by a low pH and this prevents the formation of excess lactate and/or lactic acidosis (sudden drop in blood pH). PFK catalyses an irreversible step in glycolysis.

***Practical importance of lactate formation*:** This fermentive process also occurs in also occurs in some bacteria and some fungi. It is this type of bacteria that converts lactose into lactic acid in yogurt, giving it its sour taste.

ALCOHOL FERMENTATION

Yeast and other microorganisms ferment glucose to ethanol and CO_2, rather than to lactate. Glucose is converted to pyruvate by glycolysis, and the pyruvate is converted to ethanol and CO_2 in a two-step process:

1. In the first step, pyruvate is decarboxylated in an irreversible reaction catalyzed by *pyruvate decarboxylase*. This reaction is a simple decarboxylation and does not involve the net oxidation of pyruvate. Pyruvate decarboxylase requires Mg^{2+} and has a tightly bound coenzyme, thiamine pyrophosphate.

2. In the second step, acetaldehyde is reduced to ethanol through the action of *alcohol dehydrogenase*, with the reducing power furnished by NADH derived from the dehydrogenation of glyceraldehyde 3-phosphate.. Ethanol and CO_2 are thus the end products of ethanol fermentation, and the overall equation is:

   Reaction 1: $CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2$

   Reaction 2: $CH_3CHO + NADH \rightarrow C_2H_5OH + NAD^+$

***Practical importance of alcohol fermentation*:** It is important in bread-making, brewing, and wine-making. Usually only one of the products is desired; in bread-making, the alcohol is baked out, and, in alcohol production, the carbon dioxide is released into the atmosphere or used for carbonating the beverage. When the ferment has a high concentration of pectin, minute quantities of methanol can be produced.

EVOLUTION OF FERMENTATION

Fermentation is thought to have been the primary means of energy production in earlier organisms before oxygen was at high concentration in the atmosphere, and thus would represent a more ancient form of energy production in cells.

The earliest cells lived in an atmosphere almost devoid of oxygen and had to develop strategies for deriving energy from fuel molecules under anaerobic conditions. Most modern organisms have retained the ability to constantly regenerate NAD^+ during anaerobic glycolysis by transferring electrons from NADH to form a reduced end product such as lactate or ethanol.

THE PYRUVATE DEHYDROGENASE REACTION

In an aerobic cell, when the ATP level is low and CoA is not acylated, pyruvate generated during the glycolysis is preferentially metabolized via the the pyruvate dehydrogenase complex (PDH complex). This leads to the generation of Acetyl Co-A, which acts as the donor of acetyl group for the TCA cycle. This reaction is also called as the ***link reaction***, as it serves to link the Glycolytic process to the TCA pathway.

The pyruvate dehydrogenase complex catalyzes three related reactions:

1. Pyruvate is oxidized to an acetyl group, releasing one CO_2 molecule and considerable energy.
2. Some of this energy is captured when NAD^+ is reduced to $NADH + H^+$
3. The remaining energy is captured when the acetyl group is combined with coenzyme A, yielding acetyl CoA.

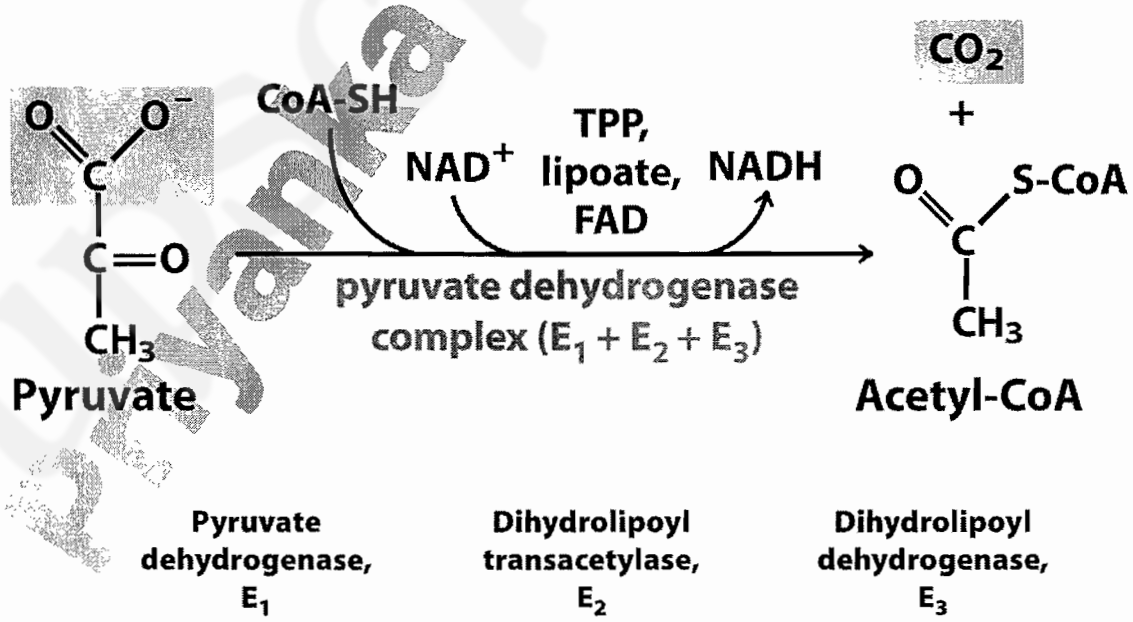
The PDH complex is comprised of multiple copies of 3 separate enzymes:

1. pyruvate dehydrogenase (20-30 copies)
2. dihydrolipoyl transacetylase (60 copies)
3. dihydrolipoyl dehydrogenase (6 copies).

The complex also requires 5 different coenzymes:

1. CoA
2. NAD+
3. FAD+
4. lipoic acid
5. thiamine pyrophosphate (TPP).

Three of the coenzymes of the complex are tightly bound to enzymes of the complex (TPP, lipoic acid and FAD) and two are employed as carriers of the products of PDH complex activity (CoA and NAD^+).



Pyruvate dehydrogenase, E_1 | **Dihydrolipoyl transacetylase, E_2** | **Dihydrolipoyl dehydrogenase, E_3**

***Reaction Mechanism*:** The first enzyme of the complex is PDH itself which oxidatively decarboxylates pyruvate. During the course of the reaction the hydroxyethyl group derived from decarboxylation of pyruvate is bound to TPP. The hydroxyethyl group is oxidated to acetyl group during the transfer to lipoic acid, the covalently bound coenzyme of lipoyl transacetylase. The transfer of the acetyl group from acyl-lipoamide to CoA results in the formation of 2 sulfhydryl (SH) groups in lipoate requiring reoxidation to the disulfide (S-S) form to regenerate lipoate as a competent acyl acceptor. The enzyme dihydrolipoyl dehydrogenase, with FAD+ as a cofactor, catalyzes that oxidation reaction. The final activity of the PDH complex is the transfer of reducing equivalents from the FADH2 of dihydrolipoyl dehydrogenase to NAD+. The fate of the NADH is oxidation via mitochondrial electron transport, to produce 2.5 equivalents of ATP.

The net result of the reactions of the PDH complex are:

$$\text{Pyruvate} + \text{CoA} + \text{NAD+} \rightarrow CO_2 + \text{Acetyl-CoA} + \text{NADH} + H^+.$$

As already mentioned, acetyl CoA generated during the PDH reaction serve as acetyl group donor in the TCA Cycle.

THE CITRIC ACID CYCLE

INTRODUCTION & OUTLINE OF THE CITRIC ACID CYCLE

Cellular respiration occurs in three major stages.

1. In the first, organic fuel molecules—glucose, fatty acids, and some amino acids—are oxidized to yield two-carbon fragments in the form of the acetyl group of acetyl-coenzyme A (acetyl-CoA).
2. In the second stage, the acetyl groups are fed into the citric acid cycle, which enzymatically oxidizes them to CO_2; the energy released is conserved in the reduced electron carriers NADH and $FADH_2$.
3. In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H^+) and electrons. The electrons are transferred to O_2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain. In the course of electron transfer, the large amount of energy released is conserved in the form of ATP, by a process called oxidative phosphorylation.

The ***citric acid cycle*** (also known as the ***tricarboxylic acid cycle***, the ***TCA cycle***, or the ***Krebs cycle***) is a series of chemical reactions of central importance in all living cells that utilize oxygen as part of cellular respiration.

The citric acid cycle is also known as the Krebs cycle after Sir Hans Adolf Krebs (1900-1981), who proposed the key elements of this pathway in 1937 and was awarded the Nobel Prize in Medicine for its discovery in 1953.

The citric acid cycle takes place within the cytoplasm in prokaryotes. Eugene Kennedy and Albert Lehninger showed in 1948 that, in eukaryotes, the entire set of reactions of the citric acid cycle takes place in mitochondria. Isolated mitochondria were found to contain not only all the enzymes and coenzymes required for the citric acid cycle, but also all the enzymes and proteins necessary for the last stage of respiration—electron transfer and ATP synthesis by oxidative phosphorylation.

The citric acid cycle is a metabolic pathway common to all aerobic cells by which acetyl group obtained from the breakdown of carbohydrates, fats and amino acids are oxidized to carbon dioxide. The initial enzyme of the cycle catalyses the condensation of a two-carbon compound acetate, with a four-carbon intermediate, oxaloacetate, to form a six-carbon compound, citric acid. The remainder of the pathway encompasses a series of oxidations and decarboxylations that regenerate the original four-carbon compound as well as $2CO_2$ and $2H_2O$. Operation of the cycle involves a stepwise removal of electrons from the series of di- and tricarboxylic intermediates. The stepwise oxidation conserves energy by storing the electrons removed in the form of NADH and $FADH_2$. These electron-rich compounds then transfer their electrons through a series of electron acceptors to oxygen, converting oxygen to H_2O. The energy available from the downhill flow of electrons to oxygen is used indirectly for the synthesis of ATP, the energy currency of the cell.

The citric acid cycle also provides precursors for many compounds such as certain amino acids, glucose (by gluconeogenetic pathway) etc. and therefore some of its reactions are therefore important even in cells performing fermentation. The TCA cycle has also been called an ***Amphibolic Pathway***.

THE PATHWAY

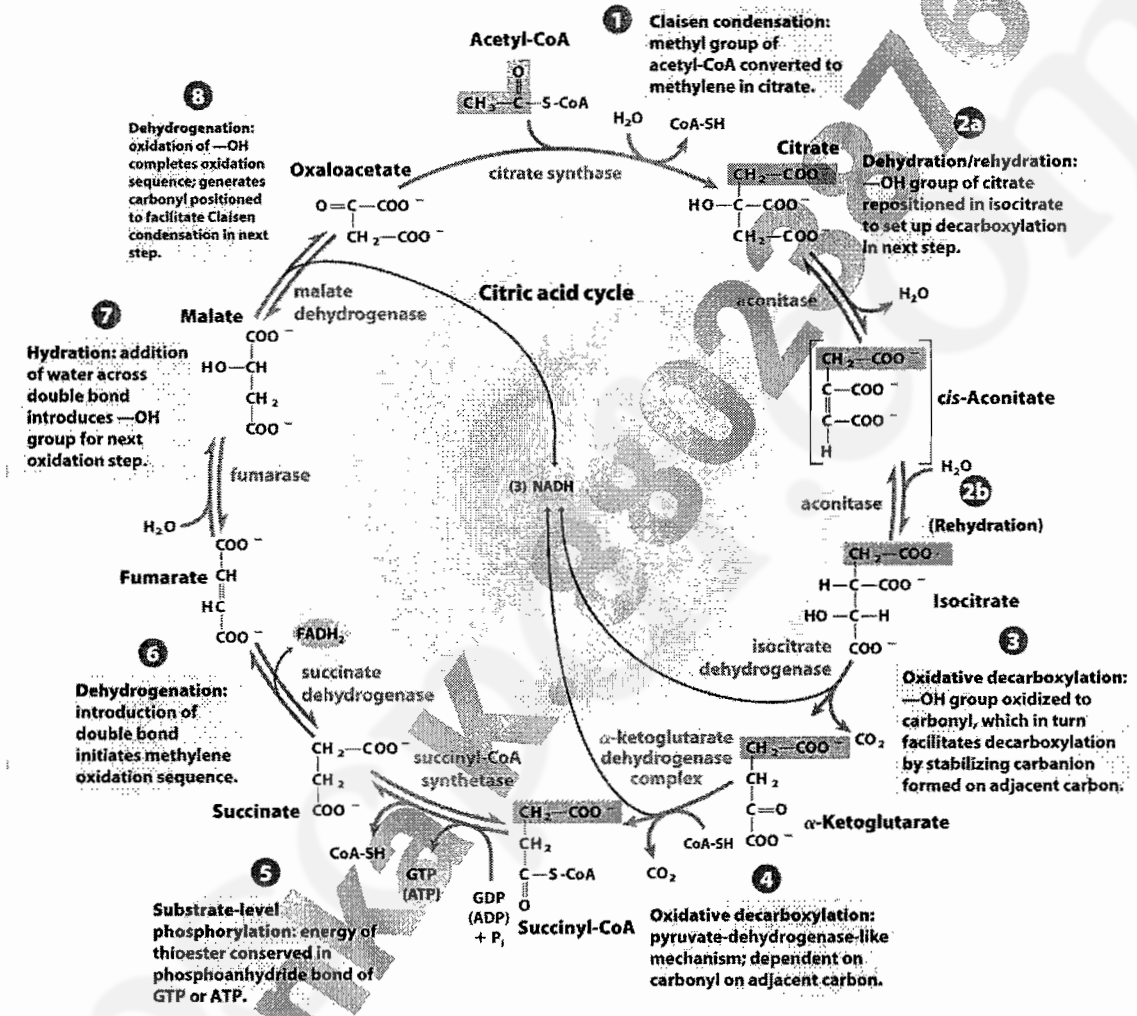


FIGURE 1: The steps of the Citric Acid Cycle

The eight reaction steps of the citric acid cycle are illustrated in Figure 1.

1. In the first committed step of the cycle, acetyl–CoA and oxaloacetate condense to form citrate in a reaction catalysed by the enzyme *citrate synthase*. The substrate of the reaction, acetyl–CoA, is formed inside the mitochondrial matrix from pyruvate, from fatty acids, or from ketogenic amino acids. The enzyme that generates acetyl–CoA from pyruvate is pyruvate dehydrogenase.

   Citrate synthase then catalyses the reaction in which the methyl group of the acetyl–CoA reacts with the ketocarbon of oxaloacetate to form citryl–CoA. Subsequent hydrolysis of citryl–CoA, generating CoA-SH, pulls the reaction in the direction of citrate.

2. In the next step, *aconitase* catalyses an isomerization of citrate to form isocitrate. During the isomerization, sequential dehydration forms cis-aconitate, and subsequent rehydration produces isocitrate. In this process, the hydroxyl group covalently linked to carbon 3 of citrate moves to carbon 2 of isocitrate.

3. *Isocitrate dehydrogenase* oxidizes and decarboxylates isocitrate in the next step of the cycle. In this two-step process, an unstable β-keto acid is first formed by removal of two hydride atoms (and two electrons) from isocitrate at the same time. NAD^+ receives the electrons and is reduced to NADH. The oxidation product, oxalosuccinate, is unstable, and loses CO_2 to form α-ketoglutarate, a five-carbon α-keto acid.

4. The reaction is an oxidative decarboxylation of α-ketoglutarate, catalysed by the enzyme *α-ketoglutarate dehydrogenase*. The substrates of this reaction are α-ketoglutarate, NAD^+ and free CoA. The reaction produces CO_2 (derived from the carbon in the 5-position of α-ketoglutarate), succinyl–CoA, the thioester of a four-carbon dicarboxylic acid, and NADH.

   The enzyme *α-ketoglutarate dehydrogenase* that catalyses this step is similar in many ways to pyruvate dehydrogenase.

   α-ketoglutarate dehydrogenase, like pyruvate dehydrogenase, is an organized assembly of three kinds of enzymes. There are multiple copies of a dehydrogenase component that catalyses the decarboxylation of α-ketoglutarate and requires thiamin pyrophosphate as a cofactor. The complex also includes multiple copies of a dihydrolipoyl succinyltransferase, which requires lipoamide as a cofactor. It is the first acceptor of the succinyl group formed by the decarboxylase and the lipoamide cofactor is reduced in the process. The same enzyme subunit then transfers the succinyl residues to CoA. The third enzyme, dihydrolipoyl dehydrogenase, employs FAD as a cofactor and regenerates the oxidized form of the lipoamide. The dihydrolipoyl dehydrogenase is identical to the one expressed as part of the pyruvate dehydrogenase enzyme complex. The molecular mass of the very large α-ketoglutarate dehydrogenase multienzyme complex is close to 5 million daltons.

   Unlike most of the other steps in the pathway, the overall α-ketoglutarate dehydrogenase reaction is highly exergonic and is virtually irreversible. Therefore, much of the energy generated by the oxidative decarboxylation of α-ketoglutarate is irreversibly lost as heat. The fact that succinyl–CoA, rather than succinate, is the product, means that some energy that might otherwise have been lost is instead stored in the high-energy thioester bond between the succinyl residue and CoA.

   The structural and mechanistic similarities between pyruvate dehydrogenase and α-ketoglutarate dehydrogenase are probably not an accident. There is a great deal of amino acid sequence similarity between pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Thus, the genes that encoded pyruvate dehydrogenase early in evolutionary time were duplicated and modified to produce α-ketoglutarate dehydrogenase. As described later, α-ketoglutarate dehydrogenase was the final link between the two primitive linear segments of the pathway and the link produced a cyclic pathway capable of efficiently generating NADH from the energy available from conversion of acetate to CO_2.

5. In the next step of the cycle, the energy stored in the succinyl–CoA thioester bond is utilized in the phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). The reaction is catalysed by the enzyme, *succinyl–CoA thiokinase*. In mammals, the substrates of the reaction are succinyl–CoA, GDP and phosphate; the products are GTP, succinate and free CoA. However, some bacteria express an ADP- and phosphate-dependent enzyme rather than one, which is specific for GDP and phosphate. Succinyl–CoA thiokinase catalyses the only step in the cycle that forms a high-energy phosphate bond and is an example of substrate-level phosphorylation. Since

the substrates of the reaction contain about the same amount of energy as the products, the thiokinase reaction is readily reversible.

6. In the next step of the cycle, *succinate dehydrogenase* removes two hydride atoms from the two internal (2,3) carbon atoms of succinate. This oxidation produces fumaric acid in which the 2,3 carbon atoms are connected by a double bond. The electrons pass to FAD rather than NAD: the oxidation of succinate to fumarate releases a relatively small amount of energy that would not support the reduction of NAD to NADH. However, the reaction does proceed in the oxidative direction because the cofactor is a covalently linked FAD that is never released from succinate dehydrogenase to interact freely in the matrix space. Although the other enzymes that catalyse steps in the citric acid cycle are free in the matrix or loosely bound to the mitochondrial inner membrane, succinate dehydrogenase is an integral membrane protein, like the electron transfer chain components. The electrons released from succinate flow directly to oxygen without contributing to the redox environment of the mitochondrial matrix space. The close physical association between succinate dehydrogenase, its covalently linked FAD cofactor and the membrane electron transfer proteins help to pull the reaction in the oxidative direction. Succinate dehydrogen ase would be highly reversible if it were free in the mitochondrial matrix and the covalently bound $FADH_2$ did not pass its electrons directly to the integral membrane proteins of the electron transfer chain.

7. Fumarate, the product of succinate oxidation, is released into the mitochondrial matrix space and in the next step of the cycle, the matrix enzyme *fumarase*, hydrates fumarate to form malate. The reaction inserts the elements of water across the double bond of fumarate in a stereospecific way so that only the l-isomer of malate forms.

8. Finally, the cycle oxidizes malate in order to regenerate oxaloacetate. The reaction is catalysed by the NAD-linked enzyme *malate dehydrogenase*, which is abundant in the mitochondrial matrix space. The NADH generated by the reaction is released to the mitochondrial matrix and can react freely with any of the other NAD-linked dehydrogenases present in the matrix.

THE AMPHIBOLY OF THE CITRIC ACID CYCLE

In aerobic organisms, the citric acid cycle is an amphibolic pathway, one that serves in both catabolic and anabolic processes. Besides its role in the oxidative catabolism of carbohydrates, fatty acids, and amino acids, the cycle provides precursors for many biosynthetic pathways (Fig. 2), through reactions that served the same purpose in anaerobic ancestors.

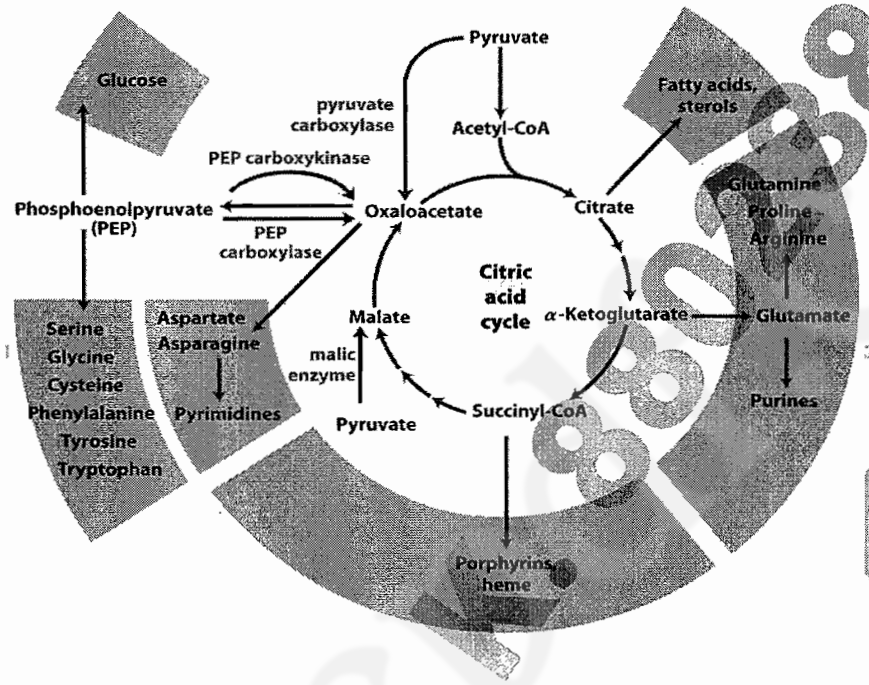
1. α-Ketoglutarate and oxaloacetate can serve as precursors of the amino acids aspartate and glutamate by simple transamination. Through aspartate and glutamate, the carbons of oxaloacetate and α-ketoglutarate are then used to build other amino acids, as well as purine and pyrimidine nucleotides.

2. Oxaloacetate is converted to glucose in gluconeogenesis.

3. Succinyl-CoA is a central intermediate in the synthesis of the porphyrin ring of heme groups, which serve as oxygen carriers (in hemoglobin and myoglobin) and electron carriers (in cytochromes)

4. Citrate produced in some organisms is used commercially for a variety of purposes The citric acid cycle is a pathway central not only to mitochondrial metabolism, but in many ways also to cellular metabolism as a whole.

In the light of mitochondrial oxidative phosphorylation, its major function is catabolic as it completely oxidizes the carbon atoms in acetyl–CoA and conserves free energy. Cycle intermediates are, however, precursors and substrates of anabolic pathways such as gluconeogenesis as well as cholesterol, fatty acid, porphyrin and protein biosynthesis. Figure 2 briefly summarizes some of the interrelationships between major mitochondrial and cytosolic metabolic pathways.



The evolutionary justification for the amphiboly comes from the fact that the citric acid cycle probably evolved in two phases.

Phase I: Origin of certain segments of the citric acid cycle in anaerobic prokaryotic bacteria as reductive biosynthetic pathways. Evidences support that enzymes for certain segments of the citric acid cycle appeared about 3 billion years ago in anaerobic prokaryotic bacteria. It happened before the accumulation of oxygen in the earth's atmosphere. Among living extant anaerobic bacteria, hyperthermophilic organisms are among the most primitive. They use segments of the citric acid cycle for reductive biosynthetic pathways. Evolutionary biochemiststs believe that biosynthesis was the original function of two large segments of the present citric acid cycle.

Two segments, one from oxaloacetate to succinate and the other from oxaloacetate to a-ketoglutarate, were originally disconnected and formed two linear noncyclic pathways. In the present-day primitive hypothermophilic bacteria and archaea, and in many anaerobic bacteria, the synthesis of succinate from oxaloacetate still occurs. In these cells, succinate is an electron sink, allowing NAD^+ to be regenerated from NADH to support glycolysis. More over succinate can participate in some amino acid biosynthetic pathways. Oxaloacetate to Succinate pathway is possible because none of the four steps involves a large energy change.

Another citric acid segment from oxaloacetate to α-ketoglutarate is also common in primitive anaerobic organisms and is probably important for the biosynthesis of glutamate, needed for protein synthesis. A simple exchange of the keto group of α-ketoglutarate for an amino group generates glutamate.

Phase II: When O_2 became available in the atmosphere, rather late in evolution; the enzyme α-ketoglutarate dehydrogenase appeared, which connected the two linear segments that had already evolved in the anaerobic environment and made possible a cyclic pathway. This enzyme is greatly similar to the PDH complex. It is widely accepted that a mutation in the PDH genes might have given rise to this new enzyme. The new cycle was running in the direction of NADH and $FADH_2$ formation.

The appearance of this cycle offered two-fold advantages:

1. The appearance of oxygen in the earth's atmosphere required an oxygen quenching mechanism
2. Much greater energy yield from the food molecule.

Since biosynthesis was the original function of two large segments of the present citric acid cycle, it is not surprising that the TCA Cycle continues to feed a number of biosynthetic pathways; thus showing an amphibolic nature. Very recently in 2004, a group of scientists discovered the reverse of the TCA cycle in some hyperthemophiles, which further support the dominant view on TCA Cycle origin.

Anaplerotic reactions replenish citric acid cycle intermediates. As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they are replenished by anaplerotic reactions. Under normal circumstances, the reactions by which cycle intermediates are siphoned off into other pathways and those by which they are replenished are in dynamic balance, so that the concentrations of the citric acid cycle intermediates remain almost constant. The most important anaplerotic reaction in mammalian liver and kidney is the reversible carboxylation of pyruvate by CO_2 to form oxaloacetate, catalyzed by pyruvate carboxylase. When the citric acid cycle is deficient in oxaloacetate or any other intermediates, pyruvate is carboxylated to produce more oxaloacetate. The enzymatic addition of a carboxyl group to pyruvate requires energy, which is supplied by ATP—the free energy required to attach a carboxyl group to pyruvate is about equal to the free energy available from ATP.

SUBSTRATE CHANNELING IN THE THE CITRIC ACID CYCLE

The eight-reaction sequence of the citric acid cycle shown in Figure 1 is an example of **substrate channeling**. The intermediates of the multistep sequence never leave the complex. Substrate channeling is the process in which the intermediate produced by one enzyme is transferred to the next enzyme without complete mixing with the bulk phase. This process is equivalent to a microcompartmentation of the intermediate, although classic diffusion occurs simultaneously to varying extents in many of these cases. This microcompartmentation and other factors of channeling provide many potential biological advantages including fas and efficient completion of biochemical processes.

Several types of evidence suggest that, in cells, multi-enzyme complexes ensure efficient passage of the product of one enzyme reaction to the next enzyme in the pathway. Such complexes are called **metabolons**.

It has also been proposed that even PDH Complex and glycolytic enzymes form multienzyme complexes for direct transfer of metabolites from the producing enzyme to the utilizing one.

REGULATION OF THE CITRIC ACID CYCLE

Regulation of the TCA cycle like that of glycolysis, occurs at both the level of entry of substrates into the cycle as well as at the key reactions of the cycle. The major regulatory events are as follows.

1. Fuel enters the TCA cycle primarily as acetyl-CoA. The generation of acetyl-CoA from carbohydrates is, therefore, a major control point of the cycle. This is the reaction catalyzed by the PDH complex. The PDH complex is inhibited by acetyl-CoA and NADH and activated by non-acetylated CoA (CoASH) and NAD^+.

2. The pyruvate dehydrogenase activities of the PDH complex are regulated by their state of phosphorylation. This modification is carried out by a specific kinase (PDH kinase) and the phosphates are removed by a specific phosphatase (PDH phosphatase). The phosphorylation of PDH inhibits its activity and, therefore, leads to decreased oxidation of pyruvate. PDH kinase is activated by NADH and acetyl-CoA and inhibited by pyruvate, ADP, CoASH, Ca^{2+} and Mg^{2+}. The PDH phosphatase, in contrast, is activated by Mg^{2+} and Ca^{2+}.

3. Since three reactions of the TCA cycle as well as PDH utilize NAD^+ as co-factor, the cellular ratio of NAD^+/NADH has a major impact on the flux of carbon through the TCA cycle.

4. Substrate availability can also regulate TCA flux. This occurs at the citrate synthase reaction because of reduced availability of oxaloacetate.

5. Product inhibition also controls the TCA flux, e.g. citrate inhibits citrate synthase, α-KGDH is inhibited by NADH and succinyl-CoA.

6. The key enzymes of the TCA cycle are also regulated allosterically by Ca^{2+}, ATP and ADP.

THE ELCTRON TRANSPORT CHAIN

The NADH and $FADH_2$ formed in glycolysis, fatty acid oxidation, and the citric acid cycle are energy-rich molecules because each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. *Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electrons from NADH or $FADH_2$ to O_2 by a series of electron carriers.* This process, which takes place in mitochondria of eukaryotic cell, and cytoplasm – Plasma Membrane interface of prokaryotic cells is the major source of ATP in aerobic organisms. For example, oxidative phosphorylation generates 26 of the 30 molecules of ATP that are formed when glucose is completely oxidized to CO_2 and H_2O.

COMPOSITION OF THE RESPIRATORY CHAIN

In eukaryotes, the respiratory chain is a series of four large protein complexes embedded in the inner mitochondrial membrane. Their properties are summarized in the table below.

Enzyme complex	Mass (kd)	Subunits	Prosthetic group	Takes e⁻ from	Gives e⁻ to
Complex I **NADH-Q oxidoreductase**	880 600 in Plants	≧34	FMN Fe-S	NADH	Quinone
Complex II **Succinate-Q reductase**	140 125 in Plants	4	FAD Fe-S	$FADH_2$	Quinone
Complex III **Q-cytochrome *c* oxidoreductase**	250 500 in Plants	10–11	Heme b_H Heme b_L Heme c_1 Fe-S	Quinone	CytC
Complex IV **Cytochrome *c* oxidase**	160 125 in Plants	10	Heme *a* Heme a_3 Cu_A and Cu_B	CytC	O_2

OPERATION OF THE RESPIRATORY CHAIN

The large transmembrane respiratory chain complexes contain multiple oxidation-reduction centers, including quinones, flavins, iron-sulfur clusters, hemes, and copper ions. Within proteins of the electron-

transport chain, electron-carrying groups are typically separated by 15 Å beyond their van der Waals contact distance. For such separations, we expect electron-transfer rates of approximately 10^4 s^{-1}, it means that the redox centres greatly facilitate electron transport.

Out of the four complexes: Three are Proton Pumps and one is a Physical Link to the Citric Acid Cycle. The four complexes are:

1. NADH-Q oxidoreductase
2. Succinate-Q reductase (the Physical Link to the Citric Acid Cycle).
3. Q-cytochrome c oxidoreductase
4. Cytochrome c oxidase.

The flow of electrons from NADH or $FADH_2$ to O_2 through protein complexes located in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix. The resulting uneven distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a proton-motive force. ATP is synthesized when protons flow back to the mitochondrial matrix through an enzyme complex. Thus, the oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane.

The following diagrams show the movement of electrons through the four complexes:

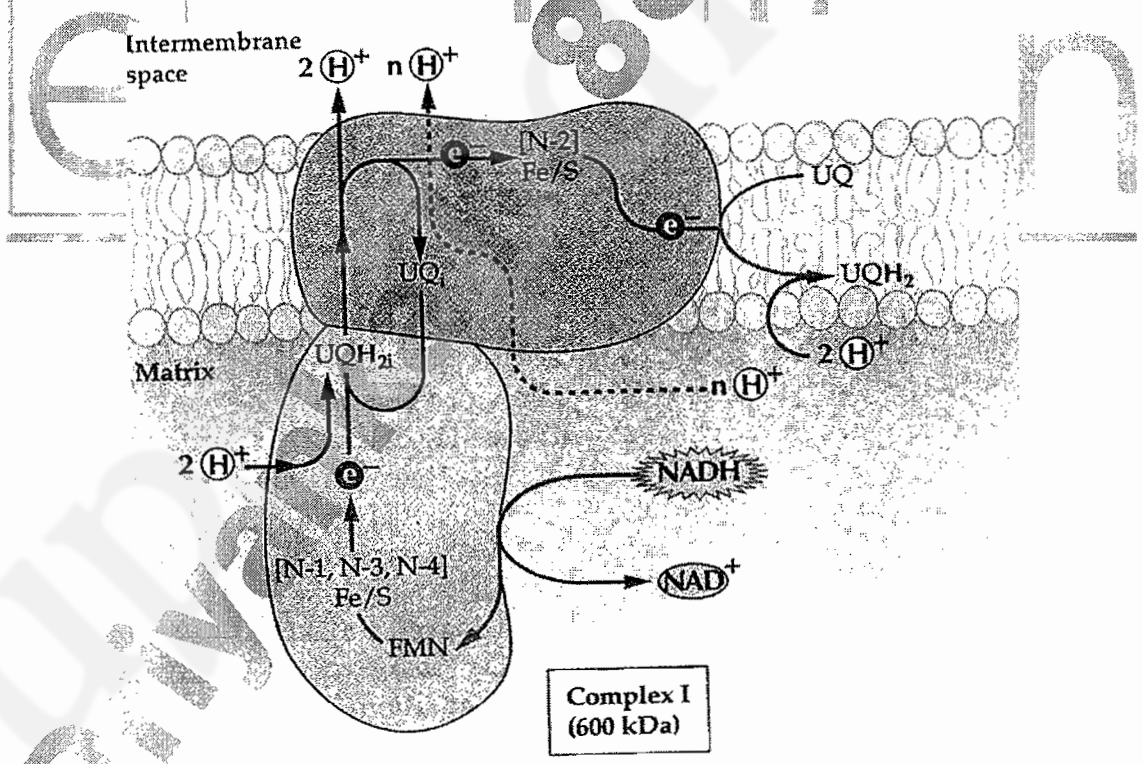


FIGURE 1: The flow of electrons through Complex I

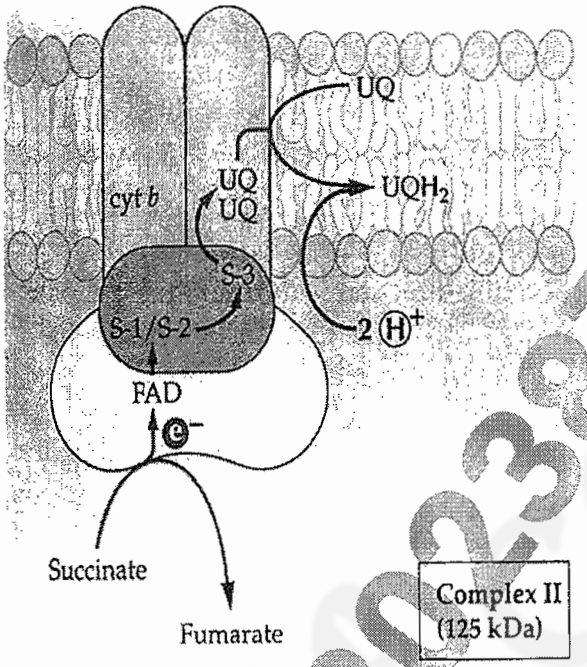


FIGURE 2: The flow of electrons through Complex II

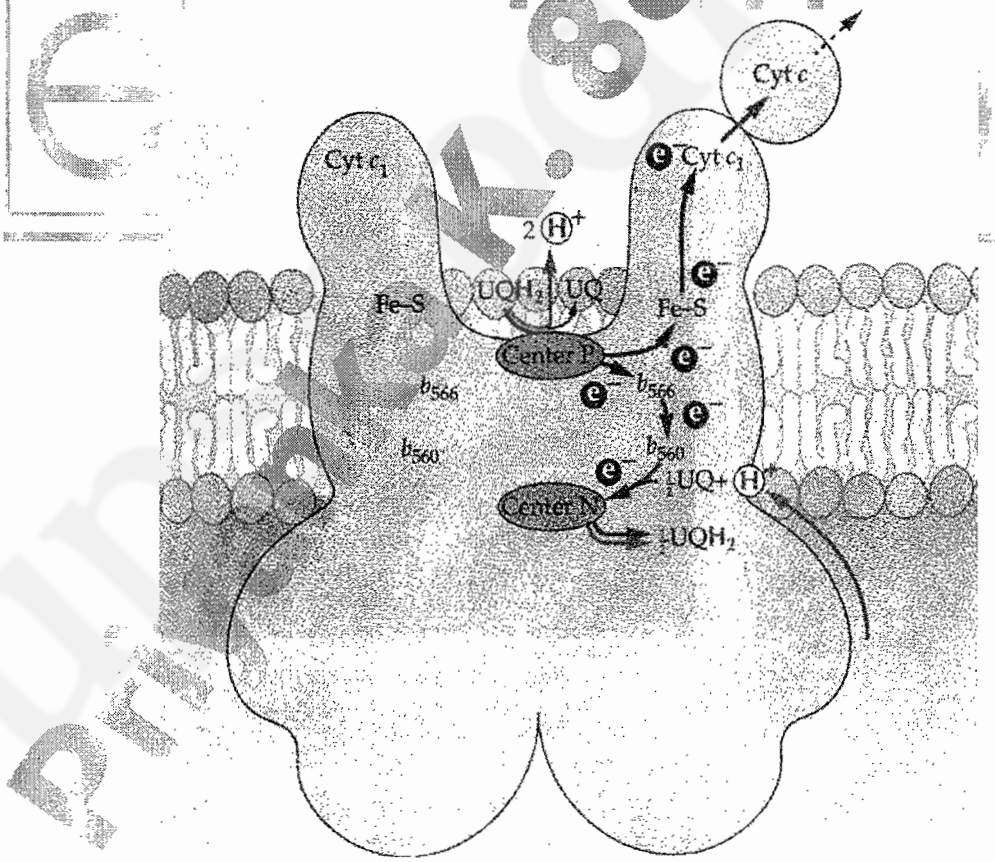


FIGURE 3: The flow of electrons through Complex III

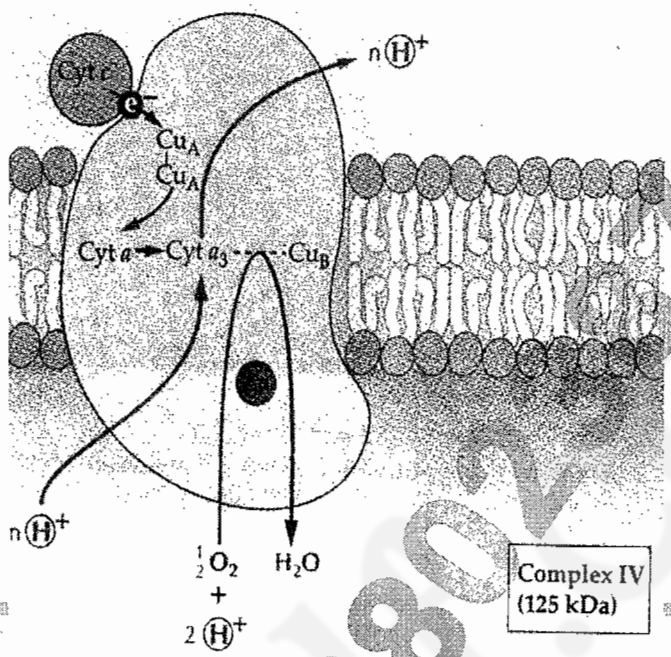


FIGURE 4: The flow of electrons through Complex IV

Complex I

Complex I (NADH dehydrogenase, also called NADH:ubiquinone oxidoreductase) removes two electrons from NADH and transfers them to a lipid-soluble carrier, *ubiquinone* (Q). The reduced product, *ubiquinol* (QH_2) is free to diffuse within the membrane. At the same time, *Complex I* moves protons (H^+) across the membrane, producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of main sites of production of a harmful free radical called superoxide.

The pathway of electrons occurs as follows:

NADH is oxidized to NAD^+, reducing Flavin mononucleotide to $FMNH_2$ in one two-electron step. The next electron carrier is a Fe-S cluster, which can only accept one electron at a time to reduce the ferric ion into a ferrous ion. In a convenient manner, $FMNH_2$ can be oxidized in only two one-electron steps, through a semiquinone intermediate. The electron thus travels from the $FMNH_2$ to the Fe-S cluster, then from the Fe-S cluster to the oxidized Q to give the free-radical (semiquinone) form of Q. This happens again to reduce the semiquinone form to the ubiquinol form, QH_2. During this process, four protons are translocated across the inner mitochondrial membrane, from the matrix to the intermembrane space. This creates a proton gradient that will be later used to generate ATP through oxidative phosphorylation.

Complex II

Complex II (succinate dehydrogenase) is not a proton pump. It serves to funnel additional electrons into the quinone pool (Q) by removing electrons from succinate and transferring them (via FAD) to Q. Complex II consists of four protein subunits: SDHA,SDHB,SDHC, and SDHD. Other electron donors (e.g., fatty acids

and glycerol 3-phosphate) also funnel electrons into Q (via FAD), again without producing a proton gradient.

Complex III

Complex III (cytochrome bc_1 complex) removes in a stepwise fashion two electrons from QH_2 at the Q_O site and sequentially transfers them to two molecules of cytochrome *c*, a water-soluble electron carrier located within the intermembrane space. The two other electrons are sequentially passed across the protein to the Q_i site where quinone is reduced to quinol. the process is called ***Q-cycle***. The bc1 complex does NOT pump protons, it helps build the proton gradient by an asymmetric absorption / release of protons.

When electron transfer is hindered (by a high membrane potential, point mutations or respiratory inhibitors such as antimycin A), Complex III may leak electrons to oxygen resulting in the formation of superoxide, a highly-toxic species, which is thought to contribute to the pathology of a number of diseases, including aging.

Complex IV

Complex IV (cytochrome *c* oxidase) removes four electrons from four molecules of cytochrome *c* and transfers them to molecular oxygen (O_2), producing two molecules of water (H_2O). At the same time, it moves four protons across the membrane, producing a proton gradient.

COUPLING WITH OXIDATIVE PHOSPHORYLATION

The chemiosmotic coupling hypothesis, as proposed by Nobel Prize in Chemistry winner Peter D. Mitchell, explains that the electron transport chain and oxidative phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane. The efflux of protons creates both a pH gradient and an electrochemical gradient. This proton gradient is used by the F_OF_1 ATP synthase complex to make ATP via oxidative phosphorylation. ATP synthase is sometimes regarded as ***complex V*** of the electron transport chain. The F_O component of ATP synthase acts as an ion channel for return of protons back to mitochondrial matrix. During their return, the free energy produced during the generation of the oxidized forms of the electron carriers (NAD^+ and Q) is released. This energy is used to drive ATP synthesis, catalyzed by the F_1 component of the complex. Coupling with oxidative phosphorylation is a key step for ATP production.

However, in certain cases, uncoupling may be biologically useful. The inner mitochondrial membrane of brown adipose tissue contains a large amount of thermogenin (an uncoupling protein), which acts as uncoupler by forming an alternative pathway for the flow of protons back to matrix. This results in consumption of energy in thermogenesis rather than ATP production. This may be useful in cases when heat production is required, for example in colds or during arise of hibernating animals. Synthetic uncouplers (e.g., 2,4-dinitrophenol) also exist, and, at high doses, are lethal.

THE COMPOSITE PICTURE OF THE RESPIRATORY CHAIN

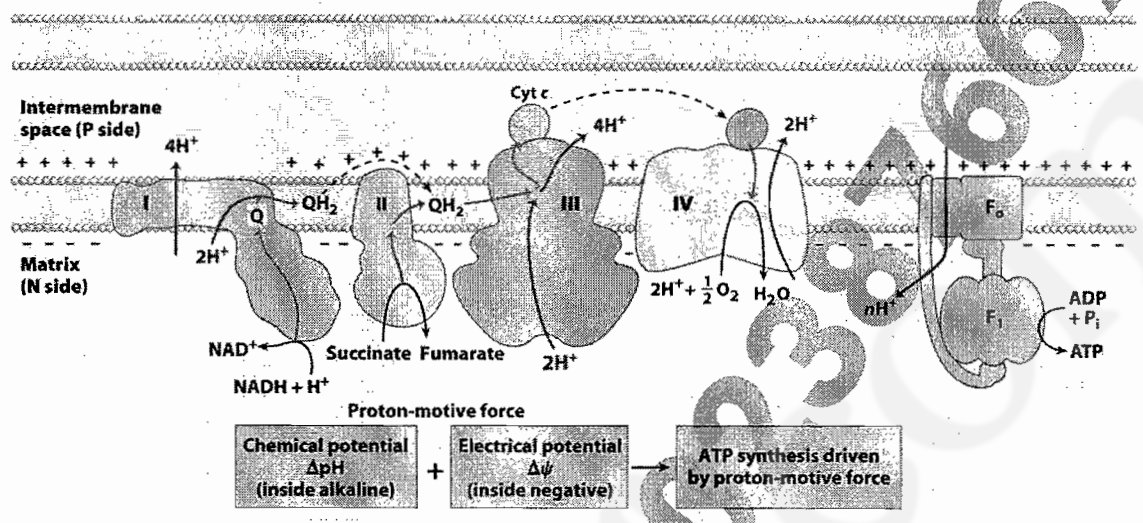


FIGURE 5: A simplified representation of the respiratory chain within the Mitochondria, with a mention of the inhibitors of the various complexes.

ATP SYNTHESIS

Two processes convert ADP into ATP:

1. Substrate-level phosphorylation
2. Chemiosmosis

SUBSTRATE-LEVEL PHOSPHORYLATION

Substrate-level phosphorylation is a type of chemical reaction that results in the formation of adenosine triphosphate (ATP) by the direct transfer of a phosphate group to adenosine diphosphate (ADP) from a reactive intermediate. In cells, it occurs primarily in the cytoplasm (in glycolysis) under both aerobic and anaerobic conditions. Unlike oxidative phosphorylation, here the oxidation & phosphorylation is not coupled.

Substrate-level phosphorylation occurs in the cytoplasm or even in mitochondria when an enzyme attaches a third phosphate to the ADP (both ADP and the phosphates are the substrates on which the enzyme acts). Substrate-level phosphorylation occurs twice in the glycolytic pathway. Besides, there are other possible ways too for substrate level phosphorylation.

1. In the pay-off phase of glycolysis, four ATP are produced by substrate-level phosphorylation: two 1,3-bisphosphoglycerate are converted to 3-phosphoglycerate by transferring a phosphate group to ADP by the enzyme phosphoglycerate kinase; and two phosphoenolpyruvate are converted to pyruvate by the transfer of their phosphate groups to ADP by the enzyme pyruvate kinase.
2. In the citric acid cycle, one guanosine triphosphate (GTP) (which can donate a phosphate group to ADP or UDP, forming the respective triphosphates is produced by substrate-level phosphorylation (per cycle, with 2 cycles per glucose molecule) when succinyl-CoA synthetase converts succinyl-CoA to succinate. This reaction occurs in the mitochondria, as does the rest of the citric acid cycle.
3. Substrate-level phosphorylation is also seen in working skeletal muscles and the brain. Phosphocreatine is stored as a readily available high-energy phosphate supply, and the enzyme creatine phosphokinase transfers a phosphate from phosphocreatine to ADP to produce ATP. Then the ATP releases giving chemical energy.

CHEMIOSMOSIS

Peter D. Mitchell proposed the chemiosmotic hypothesis in 1961. The theory suggests essentially that most ATP synthesis in respiring cells comes from the electrochemical gradient across the inner membranes of mitochondria by using the energy of NADH and $FADH_2$ formed from the breaking down of energy rich molecules such as glucose. Molecules such as glucose are metabolized to produce acetyl CoA as an energy-rich intermediate. The oxidation of acetyl CoA in the mitochondrial matrix is coupled to the reduction of a carrier molecule such as NAD and FAD. The carriers pass electrons to the electron transport chain (ETC) in the inner mitochondrial membrane, which in turn pass them to other proteins in the ETC.

The energy available in the electrons is used to pump protons from the matrix across the inner mitochondrial membrane, storing energy in the form of a transmembrane electrochemical gradient. The protons move back across the inner membrane through the enzyme ATP synthase. The flow of protons back into the matrix of the mitochondrion via ATP synthase provides enough energy for ADP to combine with inorganic phosphate to form ATP. The electrons and protons at the last pump in the ETC are taken up by oxygen to form water.

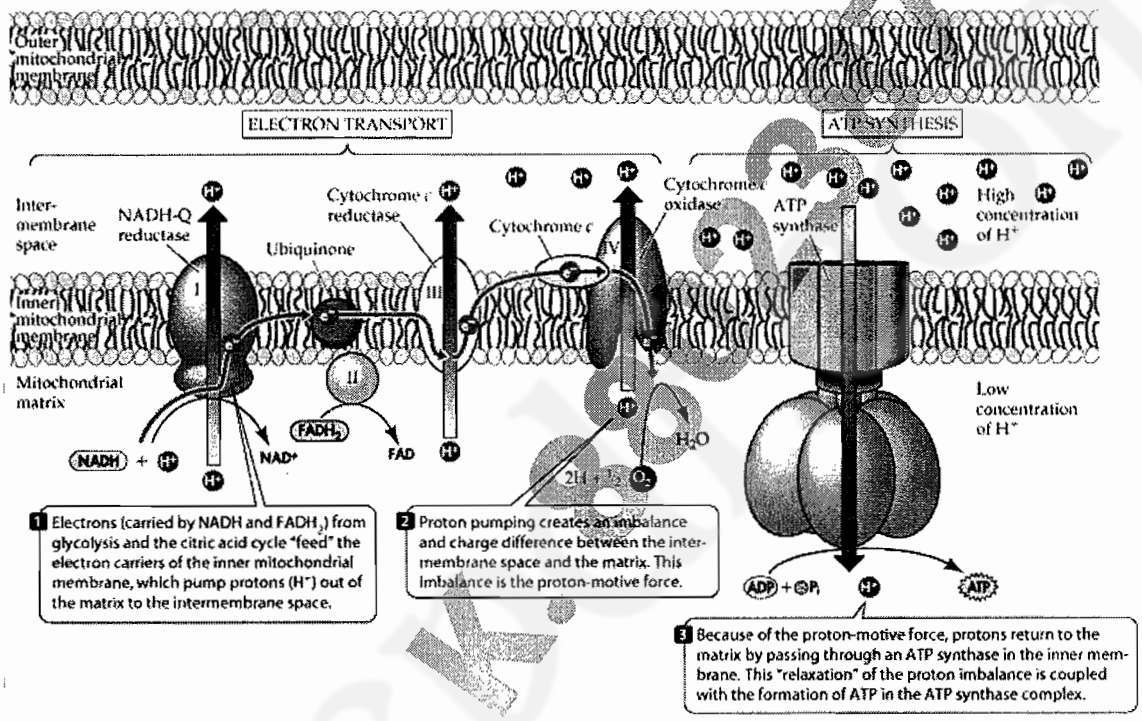


FIGURE 1: Mitochondrial Chemiosmotic Coupling

In plant cells, within the chloroplast, another type of chemiosmosis also occurs – which we call **Photophosphorylation**. The Light reactions of photosynthesis generate energy by chemiosmosis. Chlorophyll loses an electron when energized by light. This electron travels down a photosynthetic electron transport chain ending on the high energy molecule NADPH. The electrochemical gradient of Protons generated across the thylakoid membrane drives the production of ATP by ATP Synthase. This process is known as photophosphorylation.

Bacteria also can use chemiosmosis to generate ATP. Cyanobacteria, green sulfur bacteria, and purple bacteria create energy by a process called photophosphorylation. These bacteria use the energy of light to create a proton gradient using a photosynthetic electron transport chain. Some nonphotosynthetic bacteria including E. coli also contains ATP synthase.

In fact, mitochondria and chloroplasts are believed to have been formed when early eukaryotic cells ingested bacteria that could create energy using chemiosmosis. This is called the ***Endosymbiotic Theory***.

THE ATP SYNTHASE COMPLEX

The flow of electrons from NADH, and $FADH_2$ to O_2 is an exergonic process, as it becomes obvious from the following equation.

$$NADH + \tfrac{1}{2}O_2 + H^+ \rightleftharpoons H_2O + NAD^+$$
$$\Delta G^{\circ\prime} = -52.6 \text{ kcal mol}^{-1} (-220.1 \text{ kJ mol}^{-1})$$

In this section, we consider how this process is coupled to the synthesis of ATP, an endergonic process.

$$ADP + P_i + H^+ \rightleftharpoons ATP + H_2O$$
$$\Delta G^{\circ\prime} = +7.3 \text{ kcal mol}^{-1} (+30.5 \text{ kJ mol}^{-1})$$

A molecular assembly in the inner mitochondrial membrane carries out the synthesis of ATP. This enzyme complex was originally called the *mitochondrial ATPase* or *F_1F_0ATPase* because it was discovered through its catalysis of the reverse reaction, the hydrolysis of ATP. *ATP synthase,* its preferred name, emphasizes its actual role in the mitochondrion. It is also called *Complex V*.

CHEMIOSMOTIC THEORY OF MITCHELL

In 1961, PETER MITCHELL proposed that electron transport and ATP synthesis are coupled by *a proton gradient across the inner mitochondrial membrane* and *not* by a covalent high-energy intermediate or an activated protein conformation. In his model, the transfer of electrons through the respiratory chain leads to the pumping of protons from the matrix to the cytosolic side of the inner mitochondrial membrane. The H^+ concentration becomes lower in the matrix, and an electrical field with the matrix side negative is generated. Mitchell's idea, called *the chemiosmotic hypothesis,* was that this proton-motive force drives the synthesis of ATP by ATP-synthase. Mitchell's highly innovative hypothesis that oxidation and phosphorylation are coupled by a proton gradient is now supported by various experimental evidences. Indeed, electron transport does generate a proton gradient across the inner mitochondrial membrane. *The pH outside is 1.4 units lower than inside,* and the membrane potential is 0.14 V, the outside being positive. This membrane potential corresponds to a free energy of 5.2 kcal (21.8 kJ) per mole of protons.

ATP SYNTHASE ENZYME

Biochemical, electron microscopic and crystallographic studies of ATP synthase have revealed many details of its structure.

It is a large, complex membrane-embedded enzyme that looks like a ball on a stick (Figure 1). The 85 Å-diameter ball, called the F_1 subunit, protrudes into the mitochondrial matrix and contains the catalytic activity of the synthase. In fact, isolated F_1 subunits display ATPase activity. The F_1 subunit consists of *five types of polypeptide chains* (α_3, β_3, γ, δ, and ε). The α and β subunits, which make up the bulk of the F_1, are arranged alternately in a hexameric ring; they are homologous to one another and are members of the P-loop NTPase family. Both bind nucleotides but *only the β subunits participate directly in catalysis.* The central stalk consists of two proteins: γ and ε. The γ subunit includes a long α-helical coiled coil that extends into the center of the $\alpha_3\beta_3$ hexamer. *The γ subunit breaks the symmetry of the $\alpha_3\beta_3$hexamer: each*

of the β subunits is distinct by virtue of its interaction with a different face of γ. Distinguishing the three β subunits is crucial for the mechanism of ATP synthesis.

The F_0 subunit is a hydrophobic segment that spans the inner mitochondrial membrane. *F_0 contains the proton channel of the complex.* This channel consists of a ring comprising from 10 to 14 **c** subunits that are embedded in the membrane. A single **a** subunit binds to the outside of this ring. The proton channel depends on both the **a** subunit and the **c** ring. The F_0 and F_1 subunits are connected in two ways, by the central γε stalk and by an exterior column. The exterior column consists of one **a** subunit, two **b** subunits and the δ subunit. As will be discussed shortly, we can think of the enzyme as consisting of two functional components: (1) a moving unit, or *ROTOR,* consisting of the **c** ring and the γε stalk, and (2) a stationary unit, or *STATOR,* composed of the remainder of the molecule.

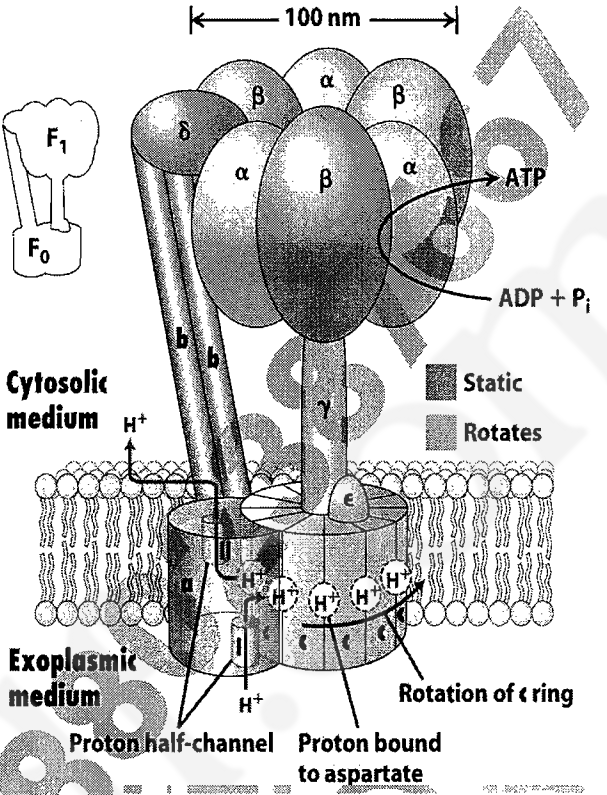


FIGURE 1: The mitochondrial ATP Synthase complex

THE BINDING-CHANGE MECHANISM OF ATP SYNTHESIS AND RELEASE OF ATP

ATP synthase catalyzes the formation of ATP from ADP and orthophosphate.

$$ADP^{3-} + HPO_4^{2-} + H^+ \rightleftharpoons ATP^{4-} + H_2O$$

The actual substrates are Mg^{2+} complexes of ADP and ATP, as in all known phosphoryl transfer reactions with these nucleotides.

The results of isotopic-exchange experiments revealed that *enzyme-bound ATP forms readily in the absence of a proton-motive force.* However, ATP does not leave the catalytic site unless protons flow through the enzyme. Thus, *the role of the proton gradient is not to form ATP but to release it from the synthase.*

Based on these and other observations, PAUL BOYER proposed a *binding-change mechanism* for proton-driven ATP synthesis (figure 2). This proposal states that changes in the properties of the three β subunits allow sequential ADP and P_i binding, ATP synthesis, and ATP release. The concepts of this initial proposal refined by more recent crystallographic and other data yield a satisfying mechanism for ATP synthesis.

As already noted, interactions with the γ subunit make the three β subunits inequivalent. One β subunit can be in the T, or tight, conformation. This conformation binds ATP with great avidity. Indeed, its affinity for

ATP is so high that it will convert bound ADP and P_i into ATP. However, the conformation of this subunit is so constrained that it cannot release ATP. A second subunit will then be in the L, or loose, conformation. This conformation binds ADP and P_i. It, too, is sufficiently constrained that it cannot release bound nucleotides. The final subunit will be in the O, or open, form. These alternating forms have been observed crystallographically.

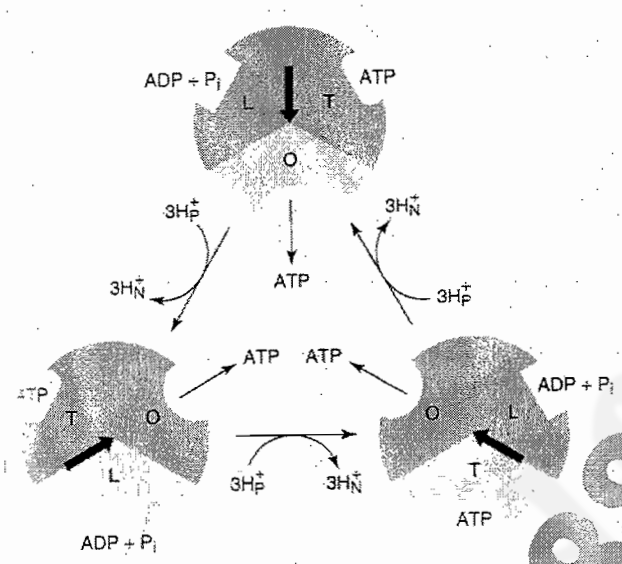


FIGURE 2: The Binding-Change Mechanism of ATP Synthesis and Release of ATP

The interconversion of these three forms can be driven by rotation of the γ subunit. The γ subunit is rotated 120° in a counterclockwise direction. This rotation will change the subunit in the T conformation into the O conformation, allowing the subunit to release the ATP that has been formed within it. The subunit in the L conformation will be converted into the T conformation, allowing the transition of bound ADP + P_i into ATP. Finally, the subunit in the O conformation will be converted into the L conformation, trapping the bound ADP and P_i so that they cannot escape. The binding of ADP and P_i to the subunit now in the O conformation completes the cycle. This mechanism suggests that ATP can be synthesized by driving the rotation of the γ subunit in the appropriate direction. Likewise, *this mechanism suggests that the hydrolysis of ATP by the enzyme should drive the rotation of the γ subunit in the opposite direction.*

ROTATIONAL CATALYSIS

Elegant experiments were performed with the use of a simple experimental system consisting of cloned $\alpha_3\beta_3\gamma$ subunits only. The β subunits were engineered to contain amino-terminal polyhistidine tags, which have a high affinity for nickel ions. This property of the tags allowed the $\alpha_3\beta_3$ assembly to be immobilized on a glass surface that had been coated with nickel ions. The γ subunit was linked to a fluorescently labeled actin filament to provide a long segment that could be observed under a fluorescence microscope. Remarkably, the addition of ATP caused the actin filament to rotate unidirectionally in a counterclockwise direction. *The γ subunit was rotating, being driven by the hydrolysis of ATP.* Thus, the catalytic activity of an individual molecule could be observed. *The γ subunit rotates in 120-degree increments.*

Proton Flow around the c Ring Powers ATP Synthesis

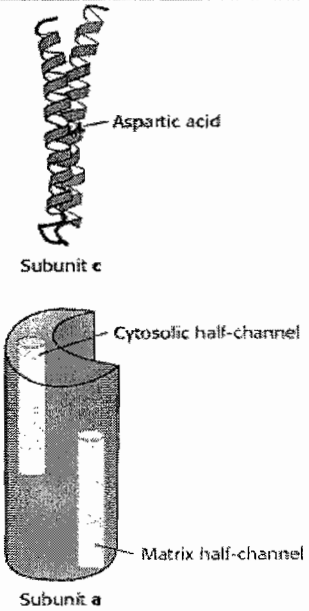


FIGURE 3: The c-protein unit and the a-protein half channels

The direct observation of rotary motion of the γ subunit is strong evidence for the rotational mechanism for ATP synthesis. The last remaining question was: How does proton flow through F_0 drive the rotation of the γ subunit? HOWARD BERG and GEORGE OSTER proposed an elegant mechanism that provides a clear answer to this question. The mechanism depends on the structures of the **a** and **c** subunits of F_0. As shown in figure 3, the structure of the **c** subunit forms a pair of α helices that span the membrane. An aspartic acid residue (*Asp 61*) is found in the middle of the second helix. When Asp 61 is in contact with the hydrophobic part of the membrane, the residue must be in the neutral aspartic acid form, rather than in the charged, aspartate form. From 10 to 14 **c** subunits assemble into a symmetric membrane-spanning ring. The **a** subunit has two *proton half-channels* that do not span the membrane. Thus, protons can pass into either of these channels, but they cannot move completely across the membrane. The **a** subunit directly adjoins the ring comprising the **c** subunits, with each half-channel directly interacting with one **c** subunit.

The Asp 61 residues of the two **c** subunits that are in contact with a half-channel give up their protons so that they are in the charged aspartate form. It is possible because they are in relatively hydrophilic environments inside the half-channel. Yet, this is an energetically unfavourable situation because there is a charged aspartate residue into the hydrophobic part of the membrane. A proton can now move through the channel that is connected to the perimitochondrial side of the membrane. (Because the proton concentration in the perimitochondrial space is more than 25 times as high on this side as on the matrix side, owing to the action of the electron-transport-chain proteins.) *For the aspartate to be protonated and come back to its neutral form, the **c** ring will now rotate.*

Thus, *protonation and deprotonation through the two half-channels yields directional rotational motion.* Each proton moves through the membrane by riding around on the rotating **c** ring to exit through the matrix half-channel.

The **c** ring is tightly linked to the γ and ε subunits. Thus, as the **c** ring turns, these subunits are turned inside the $\alpha_3\beta_3$ hexamer unit of F_1. The exterior column formed by the two **b** chains and the δ subunit prevent the $\alpha_3\beta_3$ hexamer from rotating. Thus, the proton-gradient-driven rotation of the **c** ring drives the rotation of the γ subunit, which in turn promotes the synthesis of ATP through the binding-change mechanism.

The number of **c** subunits in the **c** ring ranges between 10 and 14. This number is significant because it determines the number of protons that must be transported to generate a molecule of ATP. Each 360-degree rotation of the γ subunit leads to the synthesis and release of three molecules of ATP. Thus, if there are 10 **c** subunits in the ring (as was observed in a crystal structure of yeast mitochondrial ATP synthase), each ATP generated requires the transport of 10/3 = 3.33 protons. For simplicity, we will assume that 3 protons must flow into the matrix for each ATP formed, but we must keep in mind that the true value may differ.

TOTAL ATP GAIN IN AEROBIC RESPIRATION

REACTION SEQUENCE	ATP yield per glucose molecule
GLYCOLYSIS: CONVERSION OF GLUCOSE INTO PYRUVATE (IN THE CYTOSOL)	
Phosphorylation of glucose	- 1
Phosphorylation of fructose 6-phosphate	- 1
Dephosphorylation of 2 molecules of 1,3-BPG	+ 2
Dephosphorylation of 2 molecules of phosphoenolpyruvate	+ 2
2 molecules of NADH are formed in the oxidation of 2 molecules of glyceraldehyde 3-phosphate	
CONVERSION OF PYRUVATE INTO ACETYL COA (INSIDE MITOCHONDRIA)	
2 molecules of NADH are formed	
CITRIC ACID CYCLE (INSIDE MITOCHONDRIA)	
2 molecules of guanosine triphosphate are formed from 2 molecules of succinyl CoA	+ 2
6 molecules of NADH are formed in the oxidation of 2 molecules each of isocitrate, α-ketoglutarate, and malate	
2 molecules of $FADH_2$ are formed in the oxidation of 2 molecules of succinate	
Oxidative phosphorylation (inside mitochondria)	
2 molecules of NADH formed in glycolysis; each yields 1.5 molecules of ATP (assuming transport of NADH by the glycerol-3-phosphate shuttle)	+ 3
2 molecules of NADH formed in the oxidative decarboxylation of pyruvate; each yields 2.5 molecules of ATP	+ 5
2 molecules of $FADH_2$ formed in the citric acid cycle; each yields 1.5 molecules of ATP	+ 3
6 molecules of NADH formed in the citric acid cycle; each yields 2.5 molecules of ATP	+ 15
net yield per molecule of glucose	**+ 30**

Source: The ATP yield of oxidative phosphorylation is based on values given in P. C. Hinkle, M. A. Kumar, A. Resetar, and D. L. Harris, *Biochemistry* 30(1991):3576; Stryer, 2002, Nelson & Cox 2004

ADENOSINE TRIPHOSPHATE (ATP)

ATP [Adenosine tri Phosphate] is an adenine nucleotide that performs many essential roles in the cell.

1. It is the major energy currency of the cell, providing the energy for most of the energy-consuming activities of the cell.
2. It is one of the monomers used in the synthesis of RNA and, after conversion to deoxyATP (dATP), DNA.
3. It regulates many biochemical pathways.
4. In mammals, it also functions outside of cells.
5. Its release from damaged cells can elicit pain, and
6. Its release from the stretched wall of the urinary bladder signals when the bladder needs emptying

THE STRUCTURE OF ATP

ATP is a nucleotide consisting of an adenine, a ribose, and a triphosphate unit (Fig. 1). ATP contains the purine base adenine and the sugar ribose which together form the nucleoside adenosine. The basic building blocks used to construct ATP are carbon, hydrogen, nitrogen, oxygen, and phosphorus which are assembled in a complex that contains the number of subatomic parts equivalent to over 500 hydrogen atoms. One phosphate ester bond and two phosphate anhydride bonds hold the three phosphates (PO_4) and the ribose together. The construction also contains a β-N glycoside bond holding the ribose and the adenine together.

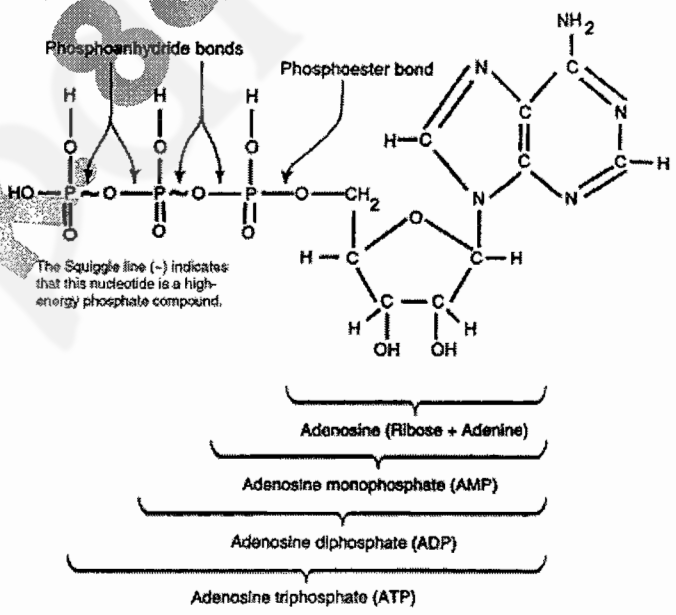


FIGURE 1: The structure of ATP

ATP AS THE UNIVERSAL CURRENCY OF FREE ENERGY IN BIOLOGICAL SYSTEMS

The process of the cell metabolism is facilitated by the use of a common energy currency, *adenosine triphosphate* (ATP), almost in the entire biological world. A part of the free energy derived from the

oxidation of food-molecules and from light is transformed into ATP, which acts as the free-energy donor in most energy-requiring processes such as motion, active transport or biosynthesis.

ATP is a nucleotide consisting of an adenine, a ribose, and a triphosphate unit. The active form of ATP is usually a complex of ATP with Mg^{2+} or Mn^{2+}. In the role of ATP as an energy carrier, its triphosphate moiety is crucial. *ATP is an energy-rich molecule because its triphosphate unit contains two phosphoanhydride bonds*. A large amount of free energy is liberated when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (P_i) or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PP_i).

$$ATP + H_2O \rightleftharpoons AMP + PP_i \qquad \Delta G^{\circ\prime} = -10.9 \text{ kcal mol}^{-1} (-45.6 \text{ kJ mol}^{-1})$$

$$ATP + H_2O \rightleftharpoons ADP + P_i \qquad \Delta G^{\circ\prime} = -7.3 \text{ kcal mol}^{-1} (-30.5 \text{ kJ mol}^{-1})$$

The free energy liberated in the hydrolysis of ATP is harnessed to drive reactions that require an input of free energy, such as muscle contraction. In turn, ATP is formed from ADP and P_i when *fuel molecules are oxidized in chemotrophs* or *when light is trapped by phototrophs*. *This ATP←→ADP cycle is the fundamental mode of energy exchange in biological systems.*

Because the amount of energy released when the phosphate bond is broken is very close to that needed by the typical biological reaction, little energy is wasted. Generally, ATP is connected to another reaction—a process called *coupling* which means the two reactions occur at the same time and at the same place, usually utilizing the same enzyme complex. Release of phosphate from ATP is exothermic (a reaction that gives off heat) and the reaction it is connected to is endothermic (requires energy input in order to occur). The terminal phosphate group is then transferred by hydrolysis to another compound, a process called *phosphorylation*, producing ADP, phosphate (P_i) and energy. ATP hydrolysis drives metabolism by shifting the equilibrium of coupled reactions. The hydrolysis of an ATP molecule in a coupled reaction then changes the equilibrium ratio of products to reactants by a very large factor, of the order of 10^8. More generally, the hydrolysis of n ATP molecules changes the equilibrium ratio of a coupled reaction (or sequence of reactions) by a factor of 10^{8n}. ATP may also be a means of conversion into an activated conformation of an inactive protein. Such a conformation can store free energy, which can then be used to drive a thermodynamically unfavorable reaction. Through such changes in conformation, molecular motors such as myosin, kinesin, and dynein convert the chemical energy of ATP into mechanical energy. Infact, this conversion is the basis of muscle contraction. ATP can also be used in the active transport of a nutrient.

ATP powers most of the energy-consuming activities of cells, such as:

1. Most anabolic reactions. Examples:
    a. joining transfer RNAs to amino acids for assembly into proteins [Link]
    b. synthesis of nucleoside triphosphates for assembly into DNA and RNA
    c. synthesis of polysaccharides
    d. synthesis of fats
2. active transport of molecules and ions

3. nerve impulses
4. maintenance of cell volume by osmosis
5. adding phosphate groups (phosphorylation) to many different proteins, e.g., to alter their activity in cell signaling.
6. muscle contraction
7. beating of cilia and flagella (including sperm)
8. bioluminescence
9. Some biosynthetic reactions are driven by hydrolysis of nucleoside triphosphates that are analogous to such as, guanosine triphosphate (GTP), uridine triphosphate (UTP), and cytidine triphosphate (CTP). It is important to note that, although all of the nucleotide triphosphates are energetically equivalent, ATP is nonetheless the primary cellular energy carrier. In addition, two important electron carriers, NAD^+ and FAD, are derivatives of ATP. Hence, *the role of ATP in energy metabolism is paramount.*

THE STRUCTURAL BASIS OF ATP AS THE FREE ENERGY CURRENCY

Phosphates are well-known high-energy molecules, meaning that comparatively high levels of energy are released when the phosphate groups are removed. Actually, the high energy content is not the result of simply the phosphate bond but the total interaction of all the atoms within the ATP molecule.

ATP has a stronger tendency to transfer its terminal phosphoryl group to water than most other tri-phosphate compounds, such as glycerol 3-phosphate. In other words, ATP has a higher *phosphoryl transfer potential (phosphoryl-group transfer potential).*

The structures of both ATP and its hydrolysis products, ADP and P_i, explain the basis of *phosphoryl transfer potential (phosphoryl-group transfer potential).* Three factors are important: *resonance stabilization, electrostatic repulsion,* and *stabilization due to hydration.* ADP and, particularly, P_i, have greater resonance stabilization than does ATP. Orthophosphate has a number of resonance forms of similar energy, whereas the γ-phosphoryl group of ATP has a smaller number (Fig. 2).

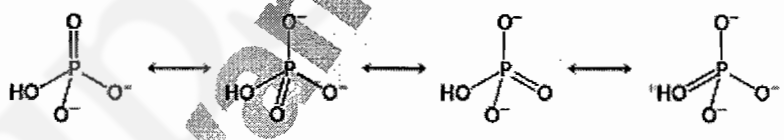


FIGURE 2: The resonance forms of similar energy, whereas the γ-phosphoryl group of ATP has a smaller number

At pH 7, the triphosphate unit of ATP carries about four negative charges. These charges repel one another because they are in close proximity. The repulsion between them is reduced when ATP is hydrolyzed. Finally, water can bind more effectively to ADP and P_i than it can to the phosphoanhydride part of ATP, stabilizing the ADP and P_i by hydration.

ATP is often called a high-energy phosphate compound, and its phosphoanhydride bonds are referred to as high-energy bonds. Indeed, a "squiggle" (~P) is often used to indicate such a bond. Nonetheless, there is

nothing special about the bonds themselves. *They are high-energy bonds in the sense that much free energy is released when they are hydrolyzed*, for the aforegiven reasons.

The standard free energies of hydrolysis provide a convenient means of comparing the phosphoryl transfer potential of phosphorylated compounds. Such comparisons reveal that ATP is not the only compound with a high phosphoryl transfer potential. In fact, some compounds in biological systems have a higher phosphoryl transfer potential than that of ATP. These compounds include phosphoenolpyruvate (PEP), 1,3-bisphosphoglycerate (1,3-BPG), and creatine phosphate.

It is significant that ATP has a phosphoryl transfer potential that is intermediate among the biologically important phosphorylated molecules. *This intermediate position enables ATP to function efficiently as a carrier of phosphoryl groups*.

BIOENERGETICS

Energy is the capacity to do work. Potential energy is the energy of state or position; it includes the energy stored in chemical bonds. Kinetic energy is the energy of motion (and related forms such as electric energy, light, and heat).

Bioenergetics is the quantitative study of the processes by which living cells use, store, and release energy. A central component of bioenergetics is energy transformation, the conversion of energy from one form to another.

In bioenergetic studies, the fundamental queries are:

1. What is the bioenergetic nature of organisms?
2. What laws govern an organism's bioenergetic dynamics?
3. Why do organisms need energy?
4. In what classes of activities do the organisms spend energy?
5. In what various ways do the organisms obtain energy from their environment?
6. The classes of organisms based on how they obtain energy from their environment
7. The activity of producer organisms as the original capturers of energy from the environment.
8. How the trophic systems move energy sources from the producer group of organisms to another?
9. How do the organisms use their sources of energy?
10. How do the organisms store / conserve energy?
11. What is the role of ATP in bioenergetics and why?

1. THE BIOENERGETIC NATURE OF ORGANISMS

There are **two fundamental facts** regarding the bioenergetic nature of the organisms.

The **biological organisms are open systems**, implying that they exchange both matter and energy with their environment. However, in this continuous exchange **the organisms and the environment are never in equilibrium**. The **organisms are always far more organized** than the environment. This phenomenon of continuous exchange of matter and energy with the environment while maintaining ones own organization is termed **dynamic steady state**.

The **organisms always act as isothermal systems**; hence, **heat is not a utilizable form of energy**. Only **chemical energy** and **solar radiational energy** can be utilized by organisms. While chemical energy can be used by all organisms (hence also called *Omnipotent Energy*), the solar radiational energy can be used only by photoautotrophs – which include plants, algae and some prokaryotes.

2. THE LAWS GOVERNING AN ORGANISM'S BIOENERGETIC DYNAMICS

The laws of thermodynamics, although originally developed from physics and chemistry, are fully valid for biological systems as well. Living things, like everything else, obey the laws of thermodynamics.

There are two laws of thermodynamics:

The first law: It describes the principle of conservation of energy. It states that *in any physical or chemical change the total amount of energy in the universe* (system + *its environment) remains constant, although the form of energy may change or energy may be transported from one region to another.* In other words, *energy cannot be created or destroyed; it may merely change its form.*

The second law: It can be stated in several ways, but simply speaking it says that *in all natural processes the entropy of the universe increases.* As a result, the quantity of energy available to do work (free energy) decreases and unusable energy (associated with entropy) increases.

The concept of Free Energy

In thermodynamics, the Gibbs free energy is a mathematical expression, developed in the 1870s by the American mathematical physicist Willard Gibbs, defined as the energy portion of a thermodynamic system available to do work. Changes in free energy, total energy, temperature, and entropy are related by the equation:

$\Delta G = \Delta H - T\Delta S$.

(Here, ΔG is free energy change; ΔH is change in enthalpy; ΔS is change in entropy and T is absolute temperature in Degree Kelvin).

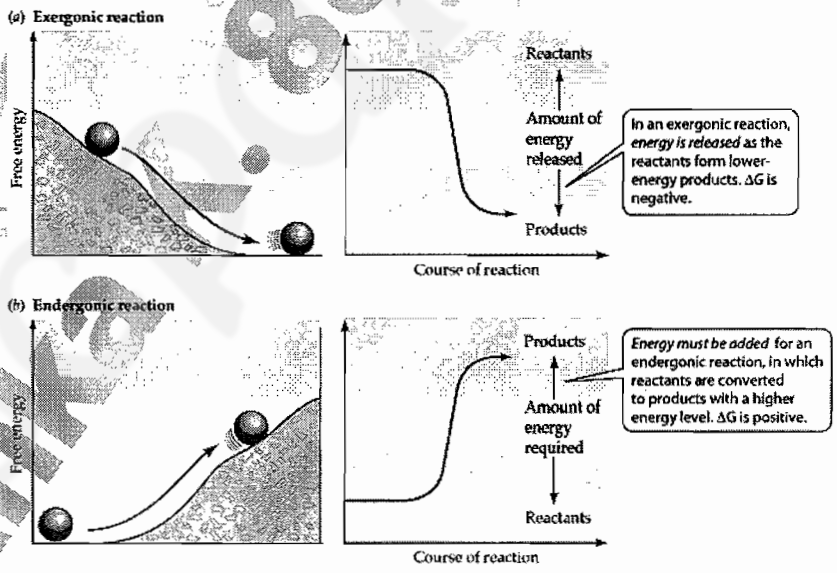


FIGURE 1: Endergonic and Exergonic Process

Based on free energy changes, there are **two types of chemical / biochemical reactions**:

1. **Exergonic reactions** release free energy and have a negative ΔG.

2. **Endergonic reactions** take up free energy and have a positive ΔG. Endergonic reactions proceed only if free energy is provided. The change in free energy (ΔG) of a reaction determines its point of chemical equilibrium, at which the forward and reverse reactions proceed at the same rate. For exergonic reactions, the equilibrium point lies toward completion (the conversion of all reactants into products).

3. WHY DO ORGANISMS NEED ENERGY?

As mentioned earlier, all biological organisms maintain a dynamic steady state with their environment. The organisms maintain a much higher degree of organization than their physical environment. For example, the atoms which constitute an organism's body are routinely exchanged with the atoms in the environment. However, the organization of atoms in the body persists even as particular atoms come and go.

This observation suggests that the organization of the organisms is far more fundamental than the material composition. **Organisms require energy from outside because energy is necessary to create and maintain their essential internal organization.**

4. CLASSES OF ACTIVITIES IN WHICH THE ORGANISMS SPEND ENERGY

Energy enables all the physiological processes to proceed, however the fundamental classes of activities include the following:

1. Maintenance of optimal physiological temperature for the cell and organism at large
2. Formation of chemical bonds in biosynthetic processes, to meet the needs of growth and repair
3. To perform mechanical works such as cell motility, mitosis, muscle contraction, flagellar and ciliary movement, DNA helix unwinding etc.
4. To perform transmembrane transports even against concentration gradient – this is an important activity to acquire nutrients and regulatory substances.
5. To generate electrical potential across membranes, which is an essential requirement in muscle contraction, nervous transmission, ATP synthesis and bacterial flagellar movement.
6. Bioluminescence in some organisms.

For these classes of activities, the chemical energy can be converted into:

1. Heat energy
2. Mechanical energy
3. Electrical energy
4. Light energy

5. VARIOUS WAYS IN WHICH ORGANISMS OBTAIN ENERGY FROM THEIR ENVIRONMENT

Living organisms derive energy from their surrounding in two ways:

1. they take up chemical fuels from the environment and extract energy by oxidizing them

2. they absorb energy from the sunlight and convert it into chemical energy

There are 3 classes of organisms based on how they obtain energy from their environment.

1. **Photoautotrophs**: They absorb energy from the sunlight and convert it into chemical energy. In this way, they synthesize their own food. Photoautotrophs include plants, algae and some prokaryotes. They are also called producers, because in ecological trophic systems they are the original sources of food for all the remaining group of organisms.

2. **Chemolithtrophs** or Chemoautotrophs are micro-organisms which take up and oxidize simple inorganic molecules in their surrounding and obtain energy from this oxidation. The liberated energy is stored as bond energy of some complex organic molecule or ATP. Although these organisms are autotrophs but they are not regarded as producers because they do not generate any significant amount of biomass to serve as other organism's food. Examples of chemolithotrophs include: *Nitrobacter, Nitrococcus, Nitrosomonas, Thiobacillus denitrificans, Bacillus denitrificans* and some species of *Pseudomonas*, *Alkaligenes* and *Bacillus*.

3. **Chemo-organotrophs** or Heterotrophs include animals, fungi and majority of the protists who obtain energy form the biomass (living / dead /decaying) generated by some other organisms. All the heterotrophs ultimately depend on the producers (plants, algae and some prokaryotes) for their food – as shown in the diagram below.

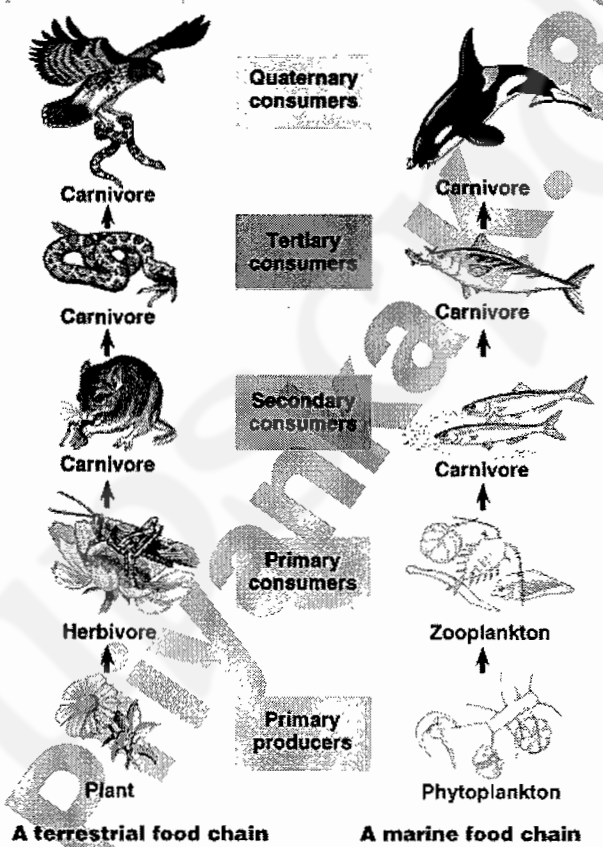


FIGURE 2: Energy transfer patterns in the members of the biota

The activity of producer organisms as the original capturers of energy from the environment.

Photosynthesizing plants, algae and prokaryotes – together called Producers – take in CO_2, water, and light energy, producing O_2 and carbohydrates. The overall reaction is:

$$6\ CO_2 + 12\ H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$$

Carbohydrates produced by photosynthesis is the *original food molecule*, because:

All other biomolecules are formed by using the reduced carbon skeleton ultimately derived from the carbohydrates

All other biomolecules are formed by using the energy liberated during respiratory oxidation of reduced carbon skeleton ultimately derived from the carbohydrates

Carbohydrates and other biomolecules synthesized using them move through the trophic system and in the process organisms of all classes get their chemical fuels as food.

How the trophic systems move energy sources

Food chains and food webs and/or trophic systems describe the feeding relationships between species in a biotic community (Fig. 2). In other words, they show the transfer of material and energy from one species to another within an ecosystem.

As usually diagrammed, an organism is connected to another organism for which it is a source of food energy and material by an arrow representing the direction of biomass transfer. As shown in the adjacent diagram, energy sources move from one group of organisms to another in a highly ordered manner – so that the energy requirements of all organisms are appropriately met.

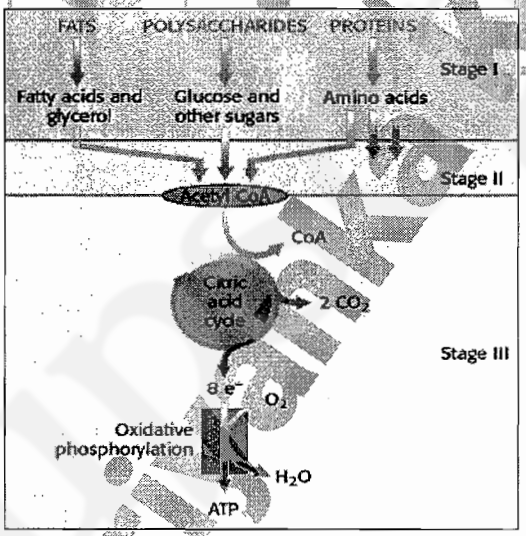
6. HOW DO THE ORGANISMS USE THEIR SOURCES OF ENERGY?

The source of energy for an organism is food.

We can define food as *a biologically utilizable organic molecule where the carbon atoms show two properties* 1. *catenation* and 2. *being extensively reduced. The food molecule serve two purposes*: 1. *a source of biological free energy that is liberated by the oxidation of food* and 2. *a source of smaller building blocks for biopolymer formation* (*required for growth and repair of the organism*).

Utilization of the food molecule as a source of energy is essentially a three step process:

1. The breakdown of large macromolecules into smaller units and finally generation of acetyl group
2. TCA Cycle
3. Electron transport and oxidative phosphorylation



7. HOW DO THE ORGANISMS STORE / CONSERVE ENERGY?

Organisms store energy in three ways:

Short term storage: When the need for energy supply is not obvious and immediate but may arise any time. ATP is the most favoured form of energy storage molecule on a short term basis.

Long term storage: Such storages are maintained to meet the requirement of emergent conditions such as starvation etc. Energy storage in this way is done through storage polymers such as Glycogen, Starch, Fats etc.

Transitional Storage: Developed in an intermediate stage of a biochemical process, when some energy transformation has to be achieved. Preferred transitional storage molecules are NADH, NADPH, and $FADH_2$. Moreover, transitional storage can also be done by establishing a proton gradient across a memrane.

8. WHAT IS THE ROLE OF ATP IN AN ORGANISMS BIOENERGETICS?

The ATP molecules generated by respiration act as the *energy currency of the cell*. It means that ATP is broken down whenever and wherever energy needs to be utilized within a cell. A large amount of free energy is liberated when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (P_i) or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PP_i).

$$ATP + H_2O \rightleftharpoons ADP + P_i \qquad \Delta G^{\circ\prime} = -7.3 \text{ kcal mol}^{-1} (-30.5 \text{ kJ mol}^{-1})$$

$$ATP + H_2O \rightleftharpoons AMP + PP_i \qquad \Delta G^{\circ\prime} = -10.9 \text{ kcal mol}^{-1} (-45.6 \text{ kJ mol}^{-1})$$

ATP powers most of the energy-consuming activities of cells, such as:

1. Most anabolic reactions. Examples:
   a. joining transfer RNAs to amino acids for assembly into proteins [Link]
   b. synthesis of nucleoside triphosphates for assembly into DNA and RNA
   c. synthesis of polysaccharides
   d. synthesis of fats
2. active transport of molecules and ions
3. nerve impulses
4. maintenance of cell volume by osmosis
5. adding phosphate groups (phosphorylation) to many different proteins, e.g., to alter their activity in cell signaling.
6. muscle contraction
7. beating of cilia and flagella (including sperm)
8. bioluminescence

ATP is often called a high-energy phosphate compound, and its phosphoanhydride bonds are referred to as high-energy bonds. Indeed, a "squiggle" (~P) is often used to indicate such a bond. *They are high-energy bonds in the sense that much free energy is released when they are hydrolyzed*, for three reasons:

1. *resonance stabilization*
2. *electrostatic repulsion*
3. *stabilization due to hydration.*

evolution

Reference Study Material

Part – II: Photosynthesis

Table of contents

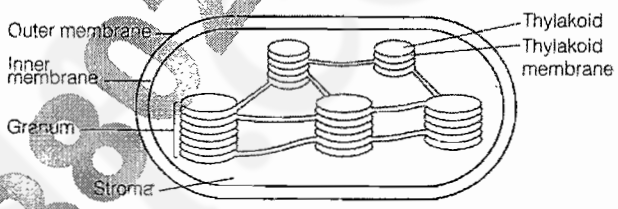
evolution
Reference Study Material

Chloroplast

INTRODUCTION TO THE PLASTIDS & CHLOROPLASTS

Plastids are characteristic of plant cells and otherwise only occur in plant-like protists like the Euglenoids. They are generally round, oval, or disc-shaped bodies about 4 to 6 micrometer in diameter and observable under the light microscope. Two unit membranes, called an *envelope,* are at the surface. Internally, plastids consist of a membrane system and matrix. *Proplastids* give rise to plastids. There are several types of plastid in plant cells.

1. **Chloroplasts** are photosynthetic plastids found in the mesophyll cells of leaves, the cortex of herbaceous stems and in small numbers elsewhere in the plant. The green coloration is due to the presence of the pigment chlorophyll. Chloroplasts are bounded by a double-membrane envelope, with the internal structure consisting of membranes and the nonmembrane area, or *stroma.* In the chloroplast, there is an elaborate structure of membranes that resemble simple, flattened sacs called *stroma lamellae.* Other membranes are more concentrated in areas of the chloroplast and form stacks of disklike, flattened sacs called *thylakoids. Grana* (singular: granum) are collections of 5 to 50 thylakoids and appear as stacks of miniature pancakes. The *grana thylakoids* are often connected to the stroma lamellae. Chloroplasts are highly organized for photosynthesis. The photosynthetic pigments are arranged in the stacks so that they can be orientated to capture as much light energy as possible.



As with the mitochondria, chloroplasts (and plastids in general) contain DNA and RNA, the latter often seen as 70S ribosomal particles. The plastid genome is circular, dsDNA like the prokaryotic chromosomes. This, together with the presence of the double outer membrane, has led to suggestions that they arose as endosymbionts – primitive photosynthetic organisms that colonized a non-photosynthetic cell. However, while some chloroplast proteins are synthesized on plastid ribosomes (70S), from genes in the chloroplast genome, many others are encoded by nuclear genes and imported.

2. **Chromoplasts** contain pigments other than chlorophyll and are associated with brightly colored structures like ripe fruit. Chromoplasts are plastids that contain carotenoid pigments only. The function of chromoplasts is obscure, but they are responsible for the coloring of autumn leaves, flowers, and fruit. In ripening fruit or fruit peel, for example, the internal membrane structure and chlorophyll of the

chloroplasts is lost while carotenoids accumulate to form the chromoplasts. A familiar example of the conversion of chloroplasts to chromoplasts is in ripening tomato berries.

3. **Leucoplasts** are colorless and are found in many cell types. Leucoplasts are nonpigmented plastids, devoid of chlorophyll and carotenoids, and are prevalent in cells of certain plant organs, including leaves, roots, and storage organs. They include **amyloplasts** that store starch and **elaioplasts** that synthesize lipid. (When plastids play an extensive role in starch biosynthesis, as in the cells of potato tubers and the endosperm of corn kernels, they are termed amyloplasts.) Leucoplasts, which also produce proteins, oils, and other substances, can develop chlorophyll and become chloroplasts upon exposure to light.
4. **Etioplasts** are an intermediate stage in the production of photosynthetic chloroplasts in tissue exposed to light for the first time.

CHLOROPLASTS

Overview

Chloroplasts are double membrane enveloped, about 5 μm long organelles found in plant cells and eukaryotic algae that conduct photosynthesis. Chloroplasts absorb sunlight and use it in conjunction with water and carbon dioxide to produce sugars. Chloroplasts capture light energy from the sun to conserve free energy in the form of ATP and reduce NADP to NADPH through a complex set of processes called photosynthesis.

Chloroplasts perform the entire primary (e.g. light capture and electron transport leading to NADPH and ATP synthesis) and most of the secondary processes (e.g. synthesis of 3-carbon phosphorylated compounds from CO_2) of photosynthesis. They also synthesize many proteins and other components.

Chloroplasts not only contain all the membrane-bound light-harvesting chlorophyll and other pigments, proteins and redox compounds involved in transport of electrons and synthesis of ATP but they also contain the soluble enzymes and substrates required for CO_2 and NO_3 assimilation by photosynthesis together with its products.

Evolutionary Origin

Chloroplasts are one of the many unique organelles in the cell, and are generally considered to have originated as endosymbiotic cyanobacteria. In this respect they are similar to mitochondria, but are found only in plants and protista. Both organelles are surrounded by a double layered composite membrane with an intermembrane space; both have their own DNA and are involved in energy metabolism; and both have reticulations, or many infoldings, filling their inner spaces.

In green plants, chloroplasts are surrounded by two lipid-bilayer membranes. The inner membrane is now believed to correspond to the outer membrane of the ancestral cyanobacterium. The chloroplast genome is considerably reduced compared to that of free-

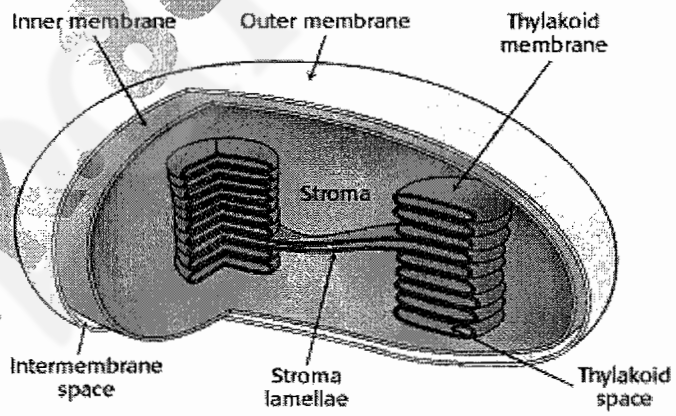
living cyanobacteria, but the parts that are still present show clear similarities. Many of the missing genes are encoded in the nuclear genome of the host. (*Please refer to a later discussion on Chloroplast Genome*).

In some algae (such as the heterokonts and other protists such as Euglenozoa and Cercozoa), chloroplasts seem to have evolved through a secondary event of endosymbiosis, in which a eukaryotic cell engulfed a second eukaryotic cell containing chloroplasts, forming chloroplasts with three or four membrane layers.

Structure: Overview

Chloroplasts are observable morphologically as flat discs usually 2 to 10 micrometer in diameter and 1 micrometer thick. The chloroplast has a two-membrane envelope termed the Inner & Outer membrane respectively. Between these two layers is the intermembrane space.

The gel state material within the chloroplast is called the stroma, which contains one or more molecules of small circular DNA. It also contains 70S ribosomes, although most of its proteins are encoded by genes contained in the cell nucleus, with the protein products transported to the chloroplast. The stroma contains soluble enzymes and substrates required for CO_2 and NO_3 assimilation by photosynthesis together with its products.



Within the stroma are stacks of thylakoids, the sub-organelles where photosynthesis actually takes place. A stack of thylakoids is called a granum (plural: grana). A thylakoid looks like a flattened disk, and inside is an empty area called the thylakoid space or lumen. The photosynthesis reaction takes place on the membrane of the thylakoid, and it involves the coupling of cross-membrane fluxes with biosynthesis via the dissipation of a proton electrochemical gradient.

Embedded in the thylakoid membrane is a dish like structure of chlorophyll molecules known as the antenna complex. This outer array helps to increase the surface area of light capture. The photons are then funneled to the centre of this complex. Two chlorophyll molecules are then ionised, producing an excited electron which then passes onto the photochemical reaction centre.

Structure: Detailed

The Envelope

Electron microscopy shows the chloroplast to consist of an envelope made up of two separate membranes, enclosing a complex of membranes, the thylakoid system. Two membranes form the envelope; each is about 5.6 nm thick and they are separated by the intra-envelope space *(ca* 10 nm), with areas of high electron density between the membranes which are possibly contact points involved in transport, for example of proteins between cytosol and stroma. The membranes are lipid bilayers of galactolipids (some 75%) with very unsaturated fatty acids (galactosyl glycerides and phosphatidyl choline), containing carotenoids but no chlorophyll. Monogalactosyldiglyceride (MGDG) makes up 50% of the membranes.

The membranes are not identical in structure or function. The outer cytoplasmic membrane allows many substrates to pass freely, whereas the inner (stromal) membrane is highly selective, allowing passage of only -some solutes by special enzyme systems called translocators. Protein particles in both membranes are complexes associated with the transporters and also with transport of other proteins.

The Stroma

The chloroplast stroma is not an homogeneous aqueous solution of small molecules and dilute proteins. In electron micrographs it contains indistinct granules and particles, which are mainly proteins, since the stroma is a dense protein gel, with about 0.4 g protein cm^{-3}. The most abundant protein is ribulose bisphosphate carboxylase/ oxygenase (RUBISCO) which forms over half of the protein; in some conditions, such as water stress or air pollution, it may form crystals.

In addition to RUBISCO, there is a large concentration of RUBISCO Activase. Also, all the other enzymes of the photosynthetic carbon reduction cycle are in the stroma, together with the enzymes and terminal redox carriers of the electron transport chain.

ATP synthase also protrudes from the thylakoids into the stroma. Other inclusions are products of the photosynthetic processes; for example, starch granules accumulate in the stroma and displace the thylakoid membranes, and globules of lipids and plastoquinone accumulate, often markedly so under stress conditions or in old leaves.

The ds, circular DNA of the chloroplast genome and mRNA plus protein-synthesizing system (70S Ribosomes) also occur in chloroplast stroma which synthesize many of their constituent proteins.

The genome of the chloroplasts found in *Marchantia polymorpha* contains 121,024 base pairs in a closed circle. These make up some 128 genes which include:

- duplicate genes encoding each of the four subunits (23S, 16S, 4.5S, and 5S) of the **ribosomal RNA** (**rRNA**) used by the chloroplast

- 37 genes encoding all the transfer RNA (tRNA) molecules used for translation within the chloroplast. Some of these are represented in the figure by black bars (a few of which are labeled).
- 4 genes encoding some of the subunits of the RNA polymerase used for transcription within the chloroplast (3 of them shown in blue)
- a gene encoding the large subunit of the enzyme **RUBISCO** (ribulose bisphosphate carboxylase oxygenase)
- 9 genes for components of photosystems I and II
- 6 genes encoding parts of the chloroplast ATP synthase
- genes for 19 of the ~60 proteins used to construct the chloroplast ribosome

All these gene products are used within the chloroplast, but all the chloroplast structures also depend on proteins

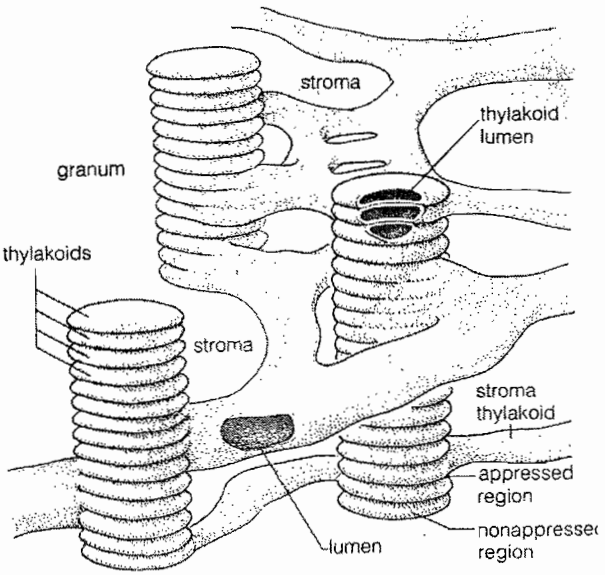
- encoded by nuclear genes
- translated in the cytosol, and
- imported into the chloroplast.

RUBISCO, for example, the enzyme that adds CO_2 to ribulose bisphosphate to start the Calvin cycle, consists of multiple copies of two subunits:

- a large one encoded in the chloroplast genome and synthesized within the chloroplast, and
- a small subunit encoded in the nuclear genome and synthesized by ribosomes in the cytosol. The small subunit must then be imported into the chloroplast.

The Thylakoid System

The most noticeable features of chloroplasts in electron micrographs are the thylakoids, which are sack-like extensive membrane vesicle system. In transverse section the thylakoids appear as parallel pairs of continuous membranes separated by a space, the thylakoid lumen, which is 5-10 nm wide. Thylakoid membranes frequently associate into granal stacks, interconnected by pairs of membranes, called stromal thylakoids (also called intergranal connections or frets), which are in contact with the stroma on both sides. The interface between the granal thylakoids are called the appressed



regions. In C3 plants over 60% of the thylakoid surface is typically in the granal organisation. The outer and end membranes of granal stacks and the stromal membranes, but not the appressed regions, have direct contact with the stroma.

The thylakoid system appears to be a single interconnecting giant closed vesicle with continuous lumen, a feature of great importance in electron transport and ATP generation. It is dynamic, changing form and relative position within the chloroplast. This may be related to the movement of materials within the chloroplast.

Thylakoid membranes are constructed of lipid with many protein complexes embedded. The composition of the thylakoid lumen is not known, but proteins of the water-splitting complex and the light-harvesting complex for example, protrude from the membranes into the lumen and occupy part of the volume.

The thylakoid membrane is a single bilayer membrane is 5-7 nm thick and consists of lipid (30% of the mass) together with proteins, pigments and other major components which are vital for photosynthesis. Thylakoid lipids are a complex mixture; some 80% is glycolipid containing galactose, such as MGDG diglyceride and digalactosyl-diglyceride (DGDG). DGDG is very important in photosynthetic membranes, and changing the proportion of DGDG in thylakoids decreases PSII efficiency and energy transfer. The fatty acids of lipids are highly unsaturated. Linolenic acid (C18:3) is the predominant fatty acid and *trans-3-hexadecanoic* acid (C16:1) acylated to phosphatidyl glycerol is specific to thylakoid membranes; its function may be structural.

Vitamin E (α-tocopherol) is a lipophilic constituent of the thylakoid membrane which may provide structure to the membrane and the protein complexes in it. However, α-tocopherol also has significant **photoprotective function**. Thylakoid membranes are subjected to intense radiation in an environment in which oxygen is produced, as well as highly energetic pigments, much reduced intermediates of electron transport and so on. They are therefore very liable to damage from, for example, reactive oxygen species, which may cause lipid peroxidation, thus destroying the membrane, or damaging protein complexes such as PSII by photoinhibition. α-tocopherol being an important antioxidant, prevents such damages.

Thylakoid membranes are particularly fluid compared with other membranes in plants; this is probably essential for the photosynthetic mechanism, with the abundant pigment-protein complexes moving within the lipid layers laterally and vertically, and also rotating. The lateral diffusion coefficient of lipids is 10^{-10} m^2 S^{-1} and that of proteins 5 X 10^{-11} m^2 S^{-1}. Distances over which pigment-protein complexes move are small (1-1000 nm) so displacements of the order of 10-100 nm occur rapidly, particularly if the proteins are charged.

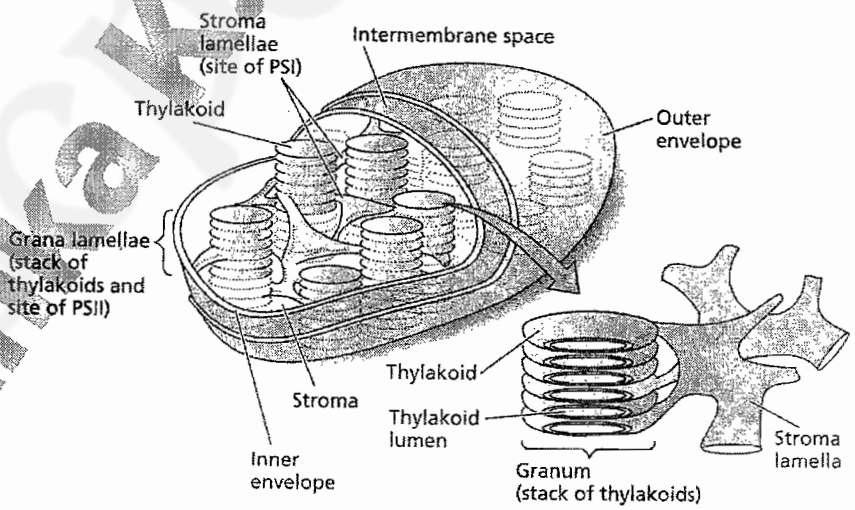
Particles or Complexes in Thylakoid Membranes

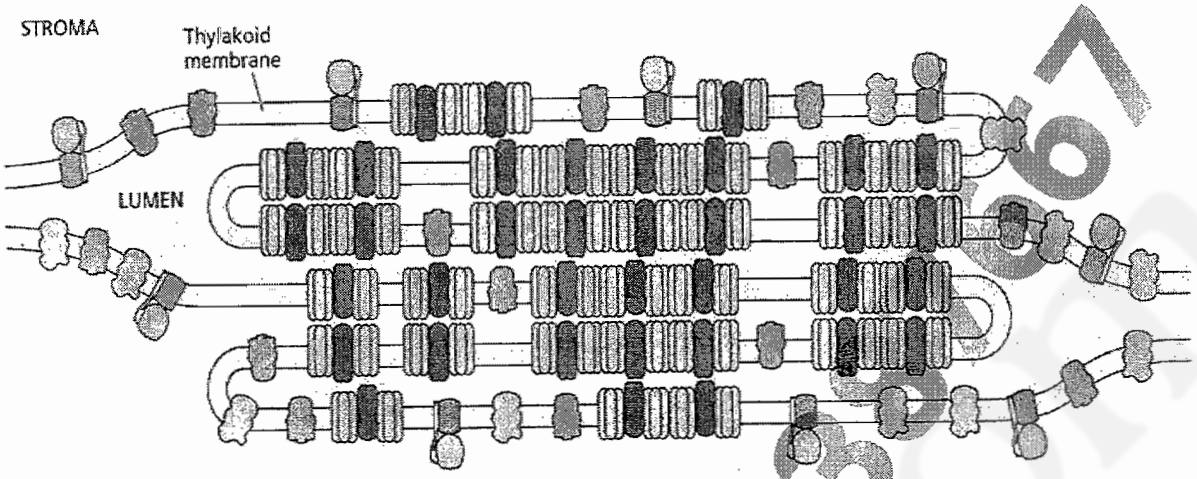
The surface structure of thylakoid membranes is observed by electron microscopy of isolated membranes and internal structure after freeze-fracturing (by cutting) frozen membranes. Particle distribution on fractured membranes has been analyzed mainly on Spinach (*Spinacea*) and it shows large particles in two populations of 15 (Pigment System 2 or PS-II) and 11 nm diameter (Pigment System 1 or PS-I), 60-70% and 30-40%, respectively.

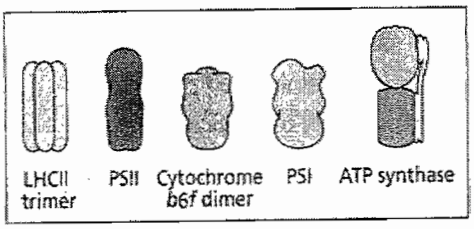
During analysis of the freeze-fractured thylakoid membranes, 4 prominent complexes are observed.

1. ATP synthase (CF1)
2. Pigment System 1 (PS-I) and the associated Light Harvesting Complex (LHC - I)
3. Pigment System 2 (PS-II) and the associated Light Harvesting Complex (LHC - II)
4. The Cytochrome b_6f complex: The *cytochrome* b_6f complex, are homologous to mitochondrial ubiquinol cytochrome *c* oxidoreductase. The cytochrome b_6f complex includes four subunits: a 23-kd cytochrome with two *b*-type hemes, a 20-kd Rieske-type Fe-S protein, a 33-kd cytochrome *f* with a *c*-type cytochrome, and a 17-kd chain.

These complexes are asymmetrically distributed, which is depicted below. This asymmetric distribution has a functional significance in the Light Reactions of Photosynthesis.







Organization of the protein complexes of the thylakoid membrane. Photosystem II is located predominantly in the stacked regions of the thylakoid membrane; photosystem I and ATP synthase are found in the unstacked regions protruding into the stroma. Cytochrome b_6f complexes are evenly distributed. This lateral separation of the two photosystems requires that electrons and protons produced by photosystem II be transported a considerable distance before they can be acted on by photosystem I and the ATP-coupling enzyme. (After Allen and Forsberg 2001.)

From the above picture, it is clear that:

1. ATP synthase (CF1) is located mostly in stromal thylakoid.
2. Pigment System 1 (PS-I) and the associated Light Harvesting Complex (LHC - I) are located mostly in stromal thylakoid.
3. Pigment System 2 (PS-II) and the associated Light Harvesting Complex (LHC - II) are located mostly in granal thylakoid's appressed region.
4. The Cytochrome b_6f complex is uniformly distributed.

The Photosystem 1

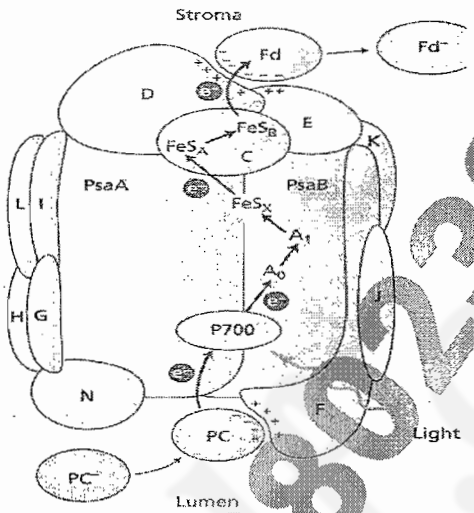
PSI is almost restricted to membranes exposed to the stroma and absent from the interior of stacked membranes. The final stage of the light reactions is catalyzed by photosystem I.

Photosystem I typically includes 13 polypeptide chains, more than 60 chlorophyll molecules, a quinone (vitamin K_1), and three 4Fe-4S clusters. The total molecular mass is more than 800 kd. The core of this system is a pair of similar subunits psaA (83 kd) and psaB (82 kd). These subunits are quite a bit larger than the core subunits of photosystem II. A special pair of chlorophyll *a* molecules lies at the center of the structure and absorb light maximally at 700 nm. This center, *P700*, initiates photoinduced charge separation.

The PSI core is now called CPC I (also called P700 chlorophyll *a* complex or chlorophyllprotein complex I, CPC I for short) and is associated with a light-harvesting chlorophyll a/b-protein

complex, now called light-harvesting complex or LHCI, composed of four different types of complexes (a-d), which has only antenna function and no photochemical activity.

The structure of PS I is depicted below. The diagram also shows the flow of electrons through the PS I.



The Photosystem 2

The PSII complex occurs in all oxygen-evolving plants (and is thus essential for 02 evolution) and contains 10% of the total chlorophyll, mainly or only chlorophyll *a* for the inner or close antenna. Probably 80% of PSII is in the appressed granaI regions, away from the stromal thylakoids.

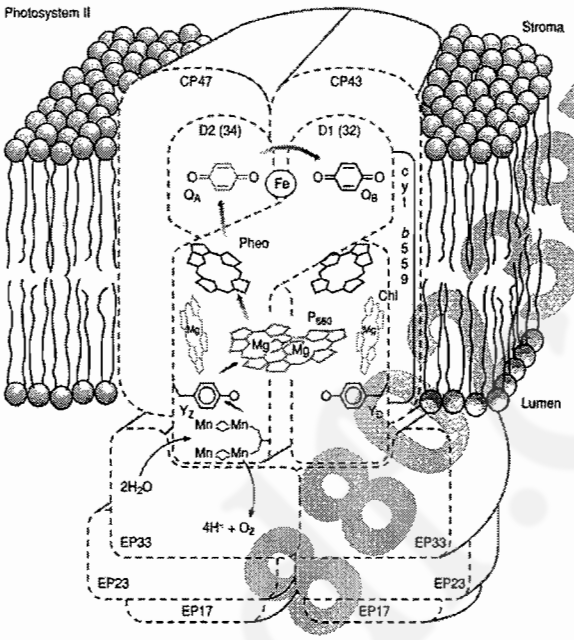
Photosystem II of green plants is reasonably similar to the bacterial reaction center. The core of photosystem II is formed by D1 and D2, a pair of similar 32-kd subunits that span the thylakoid membrane. These subunits are homologous to the L and M chains of the bacterial reaction center. Unlike the bacterial system, photosystem II contains a large number of additional subunits that bind additional chlorophylls and increase the efficiency with which light energy is absorbed and transferred to the reaction center

The PSII complex has a core of CPC II (chlorophyllprotein complex II, CPC II for short), which is probably a dimer of 230 kDa mass, each composed of two polypeptides of 50 or 60 and 70 kDa, and contains about 30-40% of the total chlorophyll *a* (no chI *b*). Photosystem II is slightly less complex but larger than the PS I. The entire PS II at least 10 polypeptide chains, more than 30 chlorophyll molecules, a nonheme iron ion, and four manganese ions.

The photochemistry of photosystem II begins with excitation of a special pair of chlorophyll molecules that are bound by the D1 and D2 subunits. This pair of molecules is analogous to the special pair in the bacterial reaction center, but it absorbs light at shorter wavelengths (maximum absorbance at 680 nm) because it consists of chlorophyll *a* molecules rather than

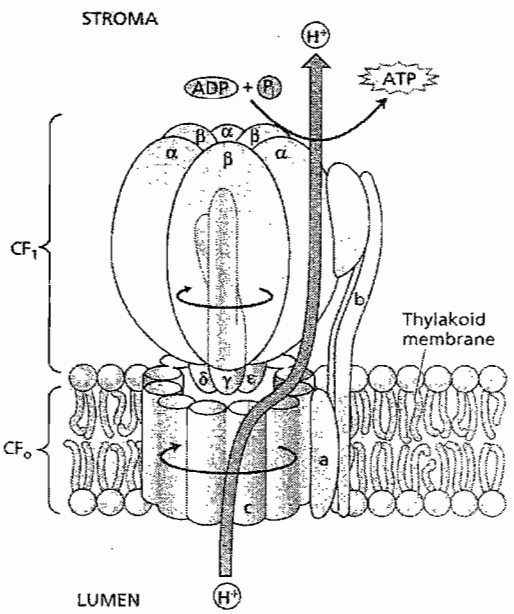
bacteriochlorophyll. The special pair is often called *P680*. The energy from the light excites an electron from its ground energy level to an excited energy level.

The structure of PS II is depicted below. The diagram also shows the flow of electrons through the PS II.



The Plastidial ATP Synthase Complex

The proton-motive force generated by the light reactions is converted into ATP by the *ATP synthase* of chloroplasts, also called the *CF_1-CF_0complex* (*C* stands for chloroplast and *F* for factor). CF_1-CF_0 ATP synthase closely resembles the F_1-F_0 complex of mitochondria.



CF_0 conducts protons across the thylakoid membrane, whereas CF_1 catalyzes the formation of ATP from ADP and P_i.

CF_0 is embedded in the thylakoid membrane. It consists of four different polypeptide chains known as I (17 kd), II (16.5 kd), III (8 kd), and IV (27 kd) having an estimated stoichiometry of 1:2:12:1. Subunits I, II, and III correspond to subunits **a, b,** and **c,** respectively, of the mitochondrial F_0 subunit, and subunit IV is similar in sequence to subunit **a.** CF_1, the site of ATP synthesis, has a subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$. The β subunits contain the catalytic sites, similar to the F_1 subunit of mitochondrial ATP synthase. *Remarkably, β*

subunits of corn chloroplast ATP synthase are more than 60% identical in amino acid sequence with those of human ATP synthase, despite the passage of approximately 1 billion years since the separation of the plant and animal kingdoms.

The membrane orientation of CF_1-CF_0 is reversed compared with that of the mitochondrial ATP synthase. Thus, protons flow *out* of the thylakoid lumen through ATP synthase into the stroma. Because CF_1 is on the stromal surface of the thylakoid membrane, the newly synthesized ATP is released directly into the stromal space.

evolution

Reference Study Material

An Overview of Photosynthesis

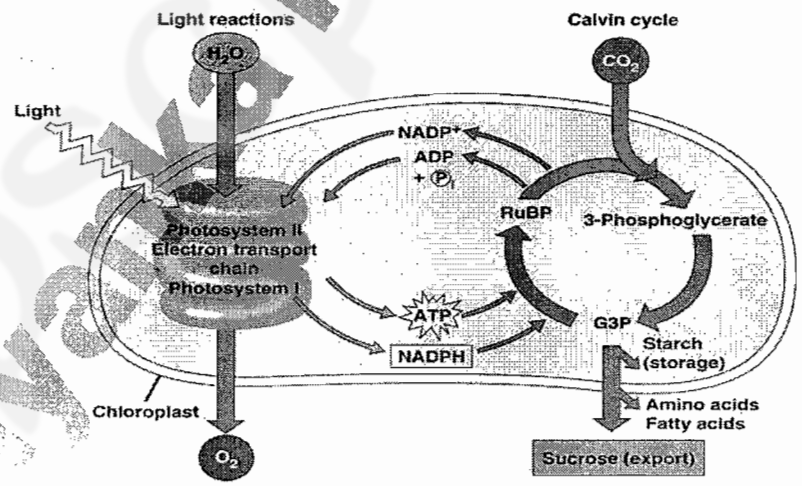
THE GENERALIZED OVERVIEW OF THE PROCESS

Photosynthesis is the physico-biochemical process by which plants, algae and photosynthetic bacteria use light energy to drive the synthesis of organic compounds. In plants, algae and certain types of bacteria, the photosynthetic process results in the release of molecular oxygen and the removal of carbon dioxide from the atmosphere that is used to synthesize carbohydrates (oxygenic photosynthesis).

Photosynthesis uses the energy of light to make the sugar, glucose. A simple general equation for photosynthesis follows.

$$6\ CO_2 + 6\ H_2O + photons \rightarrow C_6H_{12}O_6 + 6\ O_2$$

Photosynthesis occurs in two stages. In the first phase light-dependent reactions or photosynthetic reactions (also called the *Light reactions*) capture the energy of light and use it to make high-energy molecules. During the second phase, the light-independent reactions (also called the Calvin-Benson Cycle, and formerly known as the *Dark Reactions*) use the high-energy molecules to capture carbon dioxide (CO_2) and make the precursors of glucose.



Light reactions:
- Are carried out by molecules in the thylakoid membranes
- Convert light energy to the chemical energy of ATP and NADPH
- Split H_2O and release O_2 to the atmosphere

Calvin cycle reactions:
- Take place in the stroma
- Use ATP and NADPH to convert CO_2 to the sugar G3P
- Return ADP, inorganic phosphate, and NADP+ to the light reactions

In the light-dependent reactions one molecule of the pigment chlorophyll absorbs one photon and loses one electron. This electron excites pheophytin allowing the start of a flow of electrons down an electron transport chain that leads to the ultimate reduction of NADP into NADPH. In addition, it serves to create a proton gradient across the chloroplast membrane; its dissipation is used by ATP Synthase for the concomitant synthesis of ATP. The chlorophyll molecule regains the lost electron by taking one from a water molecule through a process called photolysis, that releases oxygen gas as a waste product.

In the Light-independent or dark reactions the enzyme RUBISCO captures CO_2 from the atmosphere and in a process that requires the newly formed NADPH, called the Calvin-Benson cycle releases three-carbon sugars which are later combined to form glucose.

Photosynthesis may simply be defined as the conversion of light energy into chemical energy by living organisms. It is affected by its surroundings and the rate of photosynthesis is affected by the concentration of carbon dioxide, light intensity and the temperature.

Photosynthesis Takes Place in Chloroplasts

The proteins that participate in the light reactions of photosynthesis are located in the thylakoid membranes of chloroplasts. The light reactions result in (1) the creation of reducing power for the production of NADPH, (2) the generation of a transmembrane proton gradient for the formation of ATP, and (3) the production of O_2.

Light Absorption by Chlorophyll Induces Electron Transfer

Chlorophyll molecules—tetrapyrroles with a central magnesium ion—absorb light quite efficiently because they are polyenes. An electron excited to a high-energy state by the absorption of a photon can move to nearby electron acceptors. In photosynthesis, an excited electron leaves a pair of associated chlorophyll molecules known as the special pair. The functional core of photosynthesis, a reaction center, from a photosynthetic bacterium has been studied in great detail. In this system, the electron moves from the special pair (containing bacteriochlorophyll) to a bacteriopheophytin (a bacteriochlorophyll lacking the central magnesium ion) to quinones. The reduction of quinones leads to the generation of a proton gradient, which drives ATP synthesis in a manner analogous to that of oxidative phosphorylation.

Two Photosystems Generate a Proton Gradient and NADPH in Oxygenic Photosynthesis.

Photosynthesis in green plants is mediated by two linked photosystems. In photosystem II, excitation of P680, a special pair of chlorophyll molecules located at the interface of two similar subunits, leads to electron transfer to plastoquinone in a manner analogous to that for the bacte-rial reaction center. The electrons are replenished by the extraction of electrons from water at a center containing four manganese ions. One molecule of O_2 is generated at this center for each four electrons transferred. The plastoquinol produced at photosystem II is reoxidized by the cytochrome *b6f* complex, which transfers the electrons to plastocyanin, a

soluble copper protein. From plastocyanin, the electrons enter photosystem I. In photosytem I, excitation of the special pair P700 releases electrons that flow to ferredoxin, a powerful reductant. Ferredoxin-$NADP^+$ reductase, a flavoprotein located on the stromal side of the membrane, then catalyzes the formation of NADPH. A proton gradient is generated as electrons pass through photosystem II, through the cytochrome *b6f* complex, and through ferredoxin-$NADP^+$ reductase.

A Proton Gradient Across the Thylakoid Membrane Drives ATP Synthesis

The proton gradient across the thylakoid membrane creates a proton-motive force, used by ATP synthase to form ATP. The ATP synthase of chloroplasts (also called CF_0-CF_1) closely resembles the ATPsynthesizing assemblies of bacteria and mitochondria (F_0-F_1). If the NADPH:$NADP^+$ ratio is high, electrons transferred to ferredoxin by photosystem I can reenter the cytochrome *b6f* complex. This process, called cyclic photophosphorylation, leads to the generation of a proton gradient by the cytochrome *b6f* complex without the formation of NADPH or O_2.

Accessory Pigments Funnel Energy into Reaction Centers

Light-harvesting complexes that surround the reaction centers contain additional molecules of chlorophyll *a*, as well as carotenoids and chlorophyll *b* molecules, which absorb light in the center of the visible spectrum. These accessory pigments increase efficiency in light capture by absorbing light and transferring the energy to reaction centers through resonance energy transfer. In blue-green and red algae, phycobilisomes—large protein assemblies with bound pigments called bilins—act as light-absorbing antennas.

The Calvin Cycle Synthesizes Hexoses from Carbon Dioxide and Water

ATP and NADPH formed in the light reactions of photosynthesis are used to convert CO_2 into hexoses and other organic compounds. The dark phase of photosynthesis, called the Calvin cycle, starts with the reaction of CO_2 and ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate. The steps in the conversion of 3-phosphoglycerate into fructose 6-phosphate and glucose 6-phosphate are like those of gluconeogenesis, except that glyceraldehyde 3-phosphate dehydrogenase in chloroplasts is specific for NADPH rather than NADH. Ribulose 1,5-bisphosphate is regenerated from fructose 6-phosphate, glyceraldehyde 3-phosphate, and dihydroxyacetone phosphate by a complex series of reactions. Several of the steps in the regeneration of ribulose 1,5-bisphosphate are like those of the pentose phosphate pathway. Three molecules of ATP and two molecules of NADPH are consumed for each molecule of CO_2 converted into a hexose. Starch in chloroplasts and sucrose in the cytosol are the major carbohydrate stores in plants.

The Activity of the Calvin Cycle Depends on Environmental Conditions

Reduced thioredoxin formed by the light-driven transfer of electrons from ferredoxin activates enzymes of the Calvin cycle by reducing disulfide bridges. The light-induced increase in pH and Mg^{2+} level of the stroma is important in stimulating the carboxylation of ribulose 1,5-bisphosphate by ribulose 1,5-bisphosphate carboxylase. This enzyme also catalyzes a

competing oxygenase reaction, which produces phosphoglycolate and 3-phosphoglycerate. The recycling of phosphoglycolate leads to the release of CO_2 and further consumption of O_2 in a process called photorespiration. This wasteful side reaction is minimized in tropical plants, which have an accessory pathway—called the C_4 pathway—for concentrating CO_2 at the site of the Calvin cycle. This pathway enables tropical plants to take advantage of high levels of light and minimize the oxygenation of ribulose 1,5-bisphosphate. Plants in arid ecosystems employ Crassulacean acid metabolism (CAM) to prevent dehydration. In CAM plants, the C_4 pathway is active during the night when the plant exchanges gases with the air. During the day, gas exchange is eliminated and CO_2 is generated from malate stored in vacuoles.

TYPES OF PHOTOSYNTHETIC ORGANISMS

All life can be divided into three domains, Archaea, Bacteria and Eucarya, which originated from a common ancestor . Historically, the term photosynthesis has been applied to organisms that depend on chlorophyll (or bacteriochlorophyll) for the conversion of light energy into chemical free energy. These include organisms in the domains Bacteria (photosynthetic bacteria) and Eucarya (algae and higher plants). The most primitive domain, Archaea, includes organisms known as halobacteria, that convert light energy into chemical free energy. However, the mechanism by which halobacteria convert light is fundamentally different from that of higher organisms because there is no oxidation/reduction chemistry and halobacteria cannot use CO_2 as their carbon source. Consequently some biologists do not consider halobacteria as photosynthetic. In this discussion too we shall follow the historical definition of photosynthesis and omit halobacteria.

Oxygenic Photosynthetic Organisms

The photosynthetic process in all plants and algae as well as in certain types of photosynthetic bacteria involves the reduction of CO_2 to carbohydrate and removal of electrons from H_2O, which results in the release of O_2. In this process, known as oxygenic photosynthesis, **water is oxidized by the photosystem II** reaction center, a multisubunit protein located in the photosynthetic membrane. Years of research have shown that *the structure and function of photosystem II is similar in plants, algae and certain bacteria*, so that knowledge gained in one species can be applied to others. This homology is a common feature of proteins that perform the same reaction in different species.

Anoxygenic Photosynthetic Organisms

Some photosynthetic bacteria can use light energy to extract electrons from molecules other than water. These organisms are of ancient origin, presumed to have evolved before oxygenic photosynthetic organisms. Anoxygenic photosynthetic organisms occur in the domain Bacteria and have representatives in four phyla - Purple Bacteria, Green Sulfur Bacteria, Green Gliding Bacteria, and Gram Positive Bacteria.

A Brief History of Photosynthesis Research

TIME LINE OF MAJOR BREAK THROUGH IN PHOTOSYNTHESIS STUDIES

- In the 1770s **Joseph Priestley**, an English chemist, performed experiments showing that plants release a type of air that allows combustion. Although Priestley did not know about molecular oxygen, his work showed that plants release oxygen into the atmosphere.

- Building on the work of Priestley, **Jan Ingenhousz**, a Dutch physician, demonstrated that sunlight was necessary for photosynthesis and that only the green parts of plants could release oxygen.

- During this period **Jean Senebier**, a Swiss botanist and naturalist, discovered that CO2 is required for photosynthetic growth and **Nicolas Théodore de Saussure**, a Swiss chemist and plant physiologist, showed that water is required.

- It was not until 1845 that **Julius Robert von Mayer**, a German physician and physicist, proposed that photosynthetic organisms convert light energy into chemical free energy. By the middle of the nineteenth century the key features of plant photosynthesis were known, namely, that plants could use light energy to make carbohydrates from CO2 and water.

- The empirical equation representing the net reaction of photosynthesis for oxygen evolving organisms is :

  $CO_2 + 2H_2O$ + Light Energy → $[CH_2O] + O_2 + H_2O$, where $[CH_2O]$ represents a carbohydrate (e.g., glucose, a six-carbon sugar).

  The synthesis of carbohydrate from carbon and water requires a large input of light energy. The standard free energy for the reduction of one mole of CO_2 to the level of glucose is +478 kJ/mol. Because glucose, a six carbon sugar, is often an intermediate product of photosynthesis, the net equation of photosynthesis is frequently written as:

  $6CO2 + 12H2O$ + Light Energy → $C_6H_{12}O_6 + 6O_2 + 6H_2O$

  The standard free energy for the synthesis of glucose is +2,870 kJ/mol.

  Early scientists studying photosynthesis concluded that the O_2 released by plants came from CO_2, which was thought to be split by light energy.

- In the 1930s comparison of bacterial and plant photosynthesis lead **Cornelis van Niel** to propose the general equation of photosynthesis that applies to plants, algae and photosynthetic bacteria (discussed by **Wraight**, 1982). Van Niel was aware that some photosynthetic bacteria could use hydrogen sulfide (H_2S) instead of water for photosynthesis and that these organisms released sulfur instead of oxygen. **Van Niel**, among others, concluded that photosynthesis depends on electron donation and acceptor reactions and that the O_2 released during photosynthesis comes from the oxidation of water. **Van Niel's generalized equation** is:

  $CO_2 + 2H_2A + \text{Light Energy} \rightarrow [CH_2O] + 2A + H2O.$

  In oxygenic photosynthesis, 2A is O_2, whereas in anoxygenic photosynthesis, which occurs in some photosynthetic bacteria, the electron donor can be an inorganic hydrogen donor, such as H_2S (in which case A is elemental sulfur) or an organic hydrogen donor such as succinate (in which case, A is fumarate).

- Experimental evidence that molecular oxygen came from water was provided by **Hill** and **Scarisbrick** (1940) who demonstrated oxygen evolution in the absence of CO2 in illuminated chloroplasts and by **Ruben et al.** (1941) who used ^{18}O enriched water.

- The biochemical conversion of CO_2 to carbohydrate is a reduction reaction that involves the rearrangement of covalent bonds between carbon, hydrogen and oxygen. The energy for the reduction of carbon is provided by energy rich molecules that are produced by the light driven electron transfer reactions. Carbon reduction can occur in the dark and involves a series of biochemical reactions that were elucidated by **Melvin Calvin, A. Benson** and **James Bassham** in the late 1940s and 1950s. Using the radioisotope ^{14}C, most of the intermediate steps that result in the production of carbohydrate were identified. Calvin was awarded the Nobel Prize for Chemistry in 1961 for this work.

- In 1954 **Daniel Arnon** and coworkers discovered that plants use light energy to produce ATP.

- During the same period, **Duysens** showed that the primary photochemical reaction of photosynthesis is an oxidation/reduction reaction that occurs in a protein complex (the reaction center).

- Over the next few years the work of several groups, including those of **Robert Emerson, Bessel Kok, L.N.M. Duysens, Robert Hill and Horst Witt,** combined to prove that plants, algae and cyanobacteria require two reaction centers, Photosystem II and Photosystem I, operating in series .

- In 1961, **Peter Mitchell** suggested that cells could store energy by creating a proton gradient across a membrane. Mitchell's proposal that energy is stored as an electrochemical gradient across a vesicular membrane opened the door for understanding

energy transformation by membrane systems. **He was awarded the Nobel Prize in Chemistry in 1978 for his theory of chemiosmotic energy transduction.**

- Most of the proteins required for the conversion of light energy and electron transfer reactions of photosynthesis are located in membranes. In the 1980s when **Johann Deisenhofer**, and co-workers determined the structure of the reaction center of the purple bacterium *Rhodospeudomonas viridis*. They were awarded the Nobel Prize for Chemistry in 1988 for their work, which has provided insight into the relationship between structure and function in membrane-bound proteins.

- A key element in photosynthetic energy conversion is electron transfer within and between protein complexes and simple organic molecules. The electron transfer reactions are rapid (as fast as a few picoseconds) and highly specific. Much of our current understanding of the physical principles that guide electron transfer is based on the pioneering work of Rudolph A. Marcus who received the Nobel Prize in Chemistry in 1992 for his contributions to the theory of electron transfer reaction in chemical systems.

evolution
Reference Study Material

Photosynthetic pigments

The photosynthetic products are energy-rich organic compounds. The potential chemical energy of these compounds comes from the light energy.

The light energy to be effective in photosynthesis must be absorbed by a suitable pigment. This vital role is performed by the green pigment, chlorophylls, in plants.

Synthesis of Chlorophyll

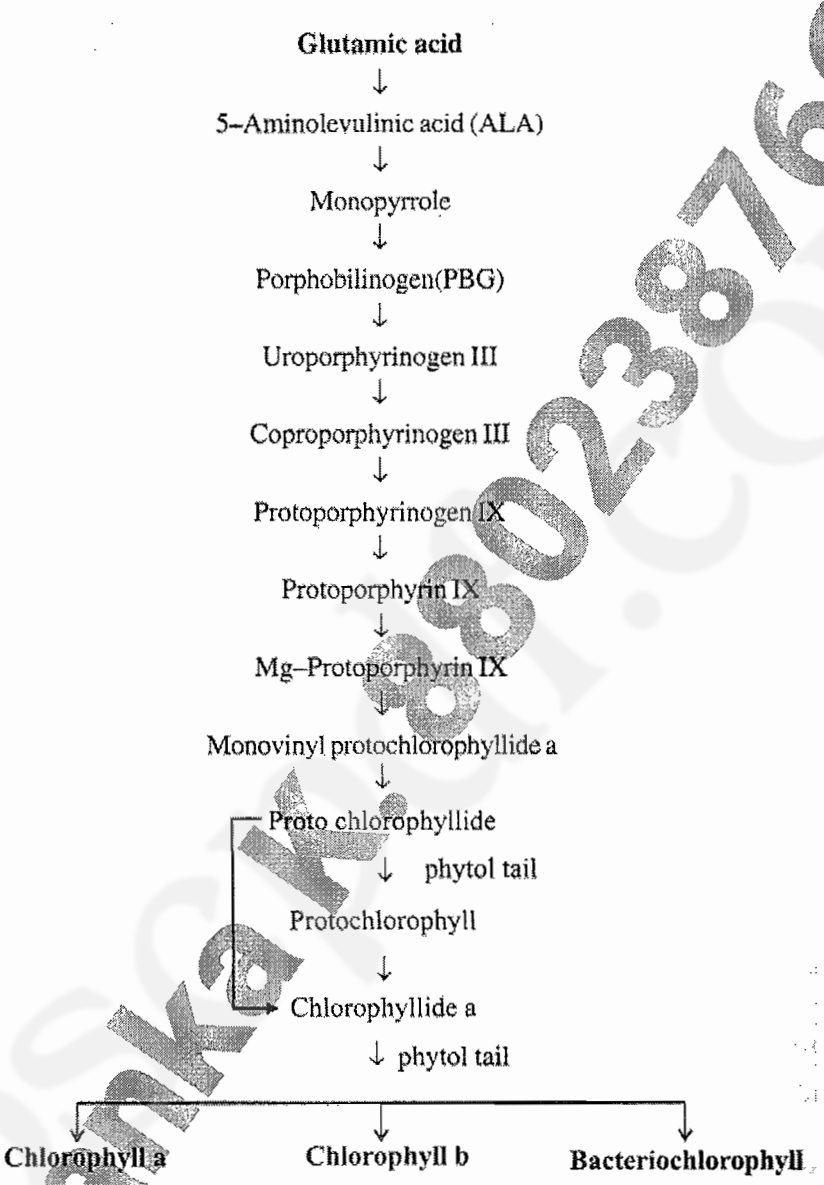
Chlorophyll is normally formed from a precursor called protochlorophyll. The latter differs from the chlorophyll in the absence of two hydrogen atoms in one of its pyrrole rings. Seedlings grown in darkness produce small amount of protochlorophyll. If such etiolated seedlings are transferred to light, the protochorophyll is quantitatively converted to chlorophyll a. Gymnosperm seedlings, however, are capable of producing chlorophyll even in darkness. In this case, the final reduction of proto chlorophyll to chlorophyll would appear to be chemical, rather than a photochemical reaction.

Granick (1954) and Shemin (1956) have explained the synthesis of chlorophyll. According to them glycine and succinyl Co-A condense to form unstable a-amino; B-ketoadipic acid, which on decarboxylation form o-aminolevulinic acid. Gassman (1967) and Bogorad (1967) have suggested that the synthesis of o-aminolevulinic acid requires the presence of light. Two molecules of o-aminolevulinic acid condense to form a monopyrrole porphobilinogen in the presence of the enzyme o-aminolevulinase (o-aminolevulinic acid dehydrase).

Four molecules of porphobilinogen give rise to uroporphyrinogen III under the influence of the enzymes uroporphyrinogen synthetase and urophyrinogen III co synthetase which act as catalysts. It is decarboxylated to coproporphyrinogen III in the presence of the enzyme uroporphyrinogen decarboxylase. It then gives rise to protoporphyrinogen IX in the presence of coproporphyrinogen III oxidated decarboxylase. Protoporphyrinogen IX is oxidised to protoporphyrin IX which incorporates magnesium to produce Mg-protoporphyrin IX. The latter takes up a methyl group from S-adenosyl methionine in the presence of the enzyme Mg-protoporphyrin methyl easterase to form Mg-protoporphyrin IX monomethyl easter. It is then converted into protochlorophyllide which takes up a phytol group to form protochlorophyll. It loses two hydrogen to become chlorophyll.

According to some the immediate precursor of chlorophyll is believed to be chlorophyllide and not the protochlorophyll. Gassman and Bogorad (1967) and Akoyunoglon and Spigelman have found that protochlorophyllide is reduced to chlorophyllide a in the presence of light.However, in gymnosperms, some ferns, and many algae light is not absolutely essential for chlorophyll synthesis. In the last step the esterification of a phytol group to chlorophyllide takes place to form chlorophyll a in the presence of the enzyme chlorophyllase.Chlorophyll a is believed to

give rise to chlorophyll **b.** The steps in the synthesis of chlorophylls and bacteriochlorophyll are given below:



Chlorophyll Pigments

There are at least seven types of chlorophylls known : **chlorophylls a, b, c, d** and **e, bacteriochlorphyll** and **bacterioviridin.** All these chlorophyll molecules contain a **tetrapyrrole** skeleton formed into a ring, with an atom of magnesium in the centre of the ring. A so-called pyrrole molecule contains a skeleton of five atoms, four of carbon and one nitrogen and the five are arranged in a ring. Four such pyrroles arranged in a ring form the 'head' of a chlorophyll molecule. Attached to this porphyrin ring at one point is an alcohol (phytol) 'tail' , a long chain oflinked carbons. Relatively minor variations in the kinds and

groupings of other atoms joined to this head and tail skeleton account for the differences among different kinds of chlorophylls.

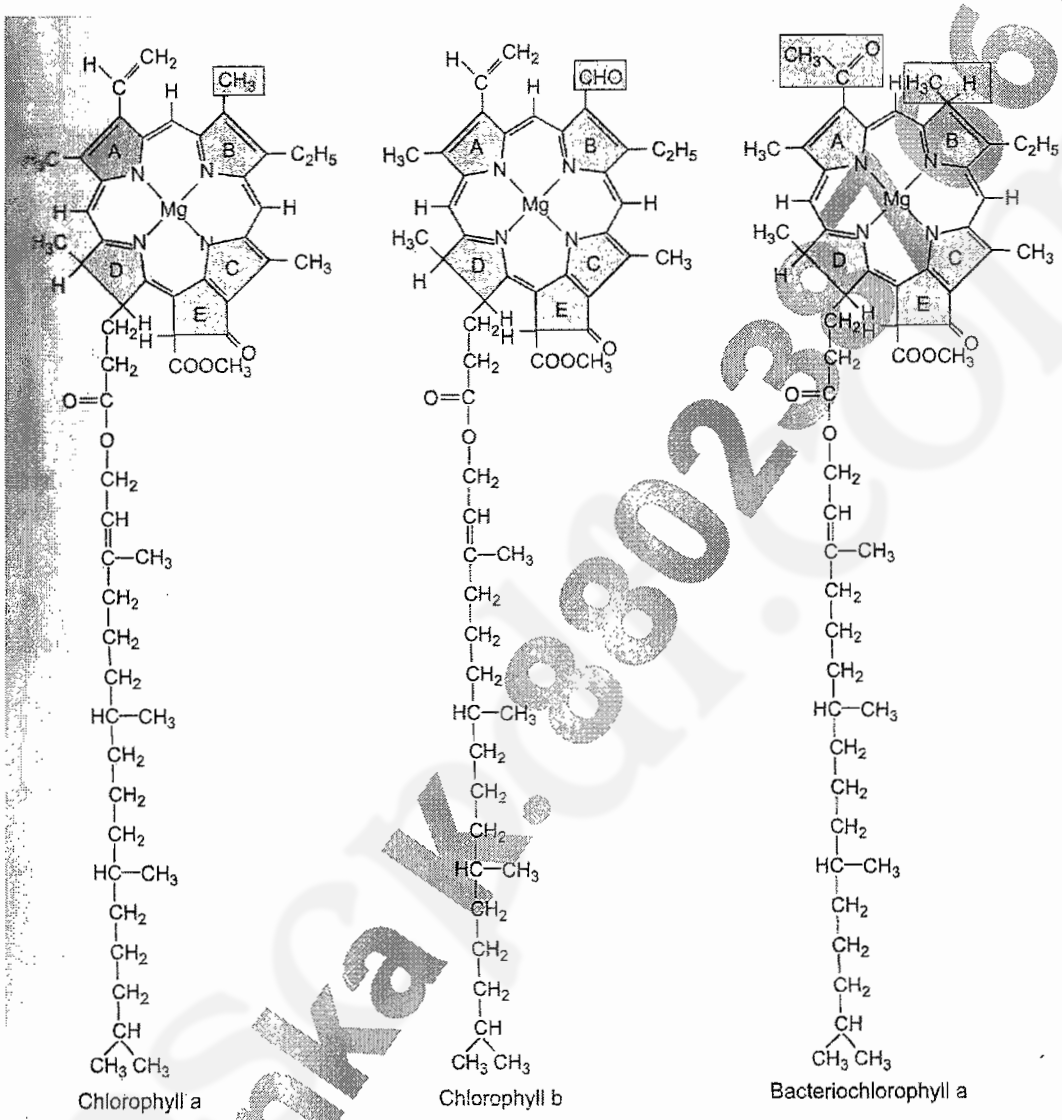


Figure 1: Structure of chlorophyll b and bacteriochlorophyll

Chlorophylls a and b are the two most abundant chlorophylls. Chlorophyll a is found in all autotrophic plants except the photosynthetic bacteria. Chlorophyll b is absent in the blue-green, brown and red algae. The other chlorophylls (c, d, e) are found only in algae and in combination with chlorophyll a. Chlorophyll a possesses - CH_3, a methyl group which is replaced by – CH0,*an* aldehyde group in chlorophyll b. The structures of chlorophyll a, chlorophyll band bacteriochlorophyll are given in Fig. 1

Both the chlorophylls a and b have hydrophilic Mg - porphyrin head and a lipophilic phytol tail.

The chlorophylls are primarily located within the grana thylakoids. The chlorophyll molecules form a monolayer between the protein and lipid layers of the membranes of the thylakoids. The hydrophilic heads of the chlorophyll molecules are embedded within the protein layer while the lipophilic tails are located within the lipid layer.

The Absorption Spectrum

The portion of the electromagnetic spectrum which participates in photosynthesis is from 300 to 900 nm. In green plants only the visible spectrum (400-750 nm) is effective in photosynthesis. Photosynthetic green bacteria can absorb wavelengths from 375-800 nm while purple photosynthetic bacteria absorb 300 to 950 nm. The chlorophyll pigments chiefly absorb in the violet blue and red parts of the spectrum. The absorption band in the violet blue region is called Soret band.

T.W. Englemann (1882) studied the effect of different regions of the visible spectrum on the rate of photosynthesis of *Spirogyra*. The efficiency of the different regions of the visible spectrum was measured on the basis of the evolution of oxygen by them. The oxygen was detected by the motile oxygen seeking (aerotactic) bacteria. The amount of oxygen released was found to be maximum in blue and red absorption bands of the chlorophyll (Fig. 2).

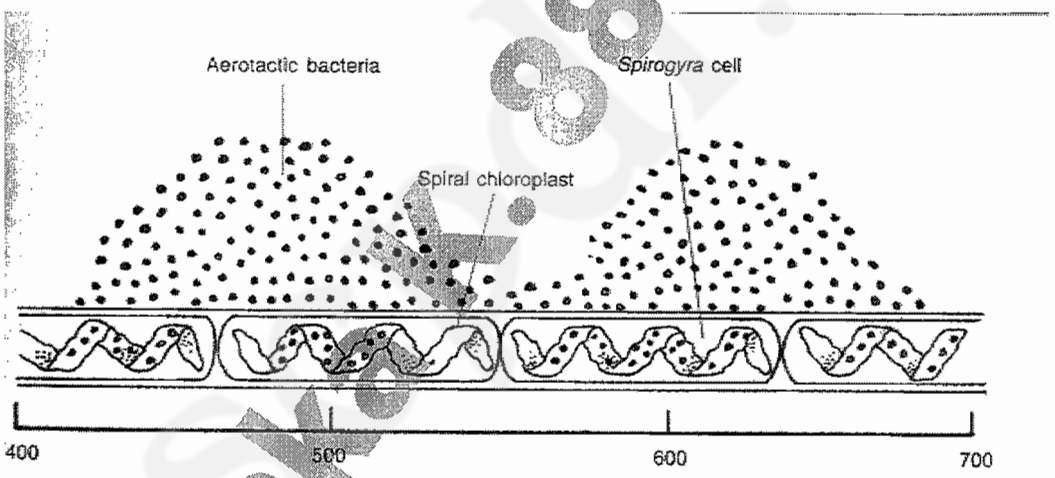


Figure 1: Englemann's experiments on filament of *Spirogyra* showing tne release of maximum oxygen in blue and red colour

The chlorophyll a has two prominent absorption peaks at **430** nm and **662** nm. There are reports of different forms of chlorophyll a with absorption peaks at **660, 670, 680, 685, 690** and **695 to 720** nanometers. These variations are perhaps due to the environmental changes. The absorption peaks for chlorophyll b occur at **453** and **642** nm (Fig.3).

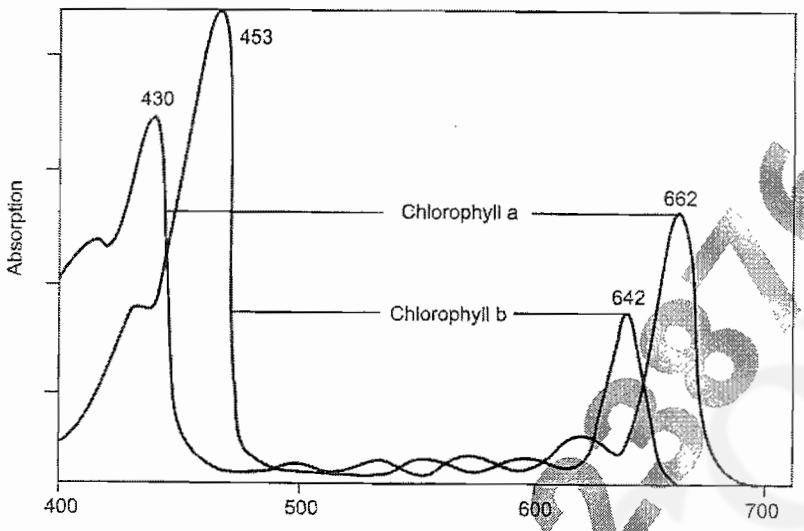


Figure 2: Absorption spectrum of chlorophyll a and chlorophyll b

The solubility properties of the two pigments differ. Chlorophyll a dissolves very well in petroleum ether while chlorophyll **b** in methyl alcohol.

The bacteriochlorophyll **a has** peak of absorption at 358 nm at 772 nm (fig. 4)

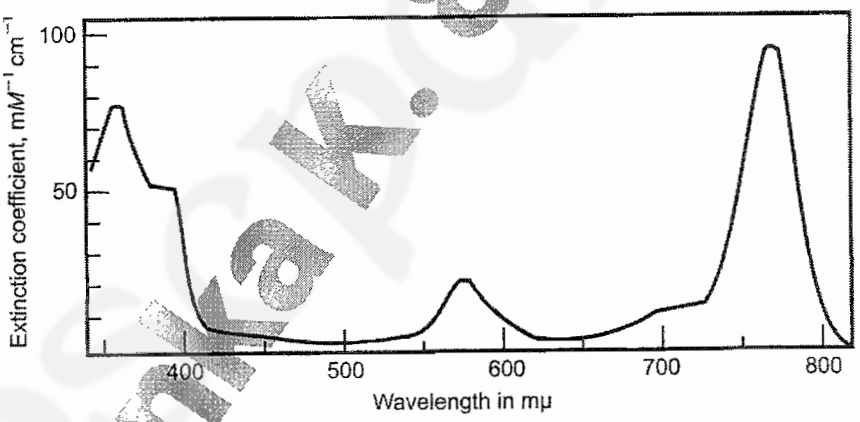


Figure 3 Absorption spectrum of bacteriochlorophyll a in ether.

The pigments in green sulphur bacteria are called **chlorobium chlorophylls 650** and **660** because its absorption peaks are in the red region at **650** and **660** nm. They are always associated with bacteriochlorophyll a. The pigment system of *Chromatium* is composed of 3 forms of bacteriochlorophyll which absorb wavelengths of **800 nm, 850** nm and **890** nm. The bacteriochlorophyll which absorbs light at 890 nm is called **B 890.** It is present in the ratio of one **B 890** to every 50 bacteriochlorophyll molecules. It is the reaction centre like **P 700** of PSI. Green sulphur bacteria has chlorophyll **770** as the reaction centre.

The strong similarity between the absorption and action spectrum of photosynthesis shows that the rate of photosynthesis is proportionate to the light energy absorbed by the chlorophyll molecules.

CAROTENOIDS

The carotenoids are the main accessory pigments in photosynthesis. They transfer the light energy to chlorophyll for photosynthesis. The carotenoids are widely distributed in plants. They occur in bacteria, algae and higher plants. They include orange **carotenes** and yellow **xanthophylls.** They absorb wavelengths **400** nm to **500** nm because of which they are orange in colour. Of the carotenes BETA carotene is the abundant type. It absorbs blue light, and, therefore, appears yellow in colour. ALPHA-carotene is present in very small amounts in certain species. The carotenoids perform two types of functions in the green plants. They trap light energy and transfer it to the chlorophyll a particularly in algae and to some extent in higher plants. In higher plants this function is performed by **lutein** of the **xanthophylls** and BETA carotene.

At high light intensities the entire cell apparatus is oxidised by atmospheric oxygen into carbon dioxide. This process is termed **photo-oxidation** which is as good as combustion. The carotenoids (BETA-carotene) protect the photosynthetic apparatus from this type of destruction by trapping and dissipating the excess excitation energy which would have otherwise converted molecular oxygen to a highly reactive and mutagenic superoxide (02-)' The dissipation of excess energy in the form of heat is facilitated by **xanthophyll** cycle.

Carotenoids consist of long chains of carbon atoms linked by conjugated single and double bonds with six-carbon rings as each end (Fig.5).

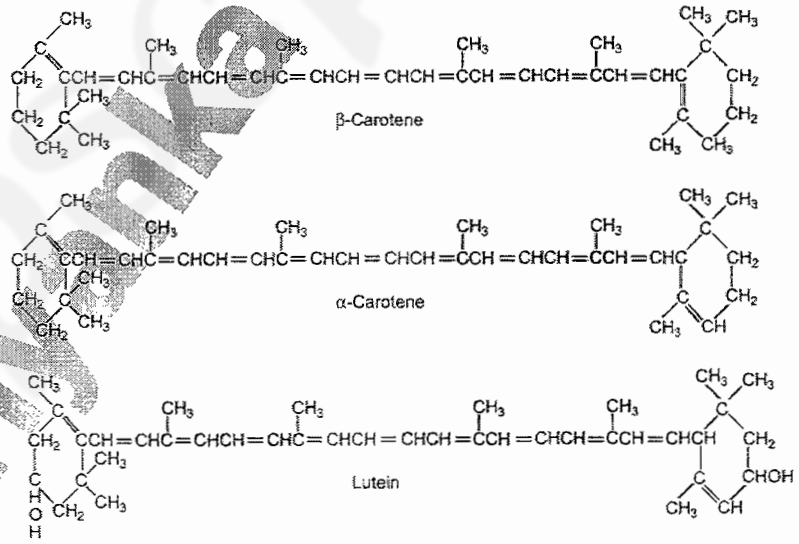


Figure 4: The molecular structures of the major carotenoids of higher plants.

The carotenes are hydrocarbons i.e. they contain carbon and hydrogen. The xanthophylls (also known as carotenols) are alcohols and ketones and contain oxygen, carbon and hydrogen. Like chlorophylls they are located in chloroplasts and also in chromoplasts. Carotenes are named after carrot in which they are abundant.

PHYCOBILINS

Engelmann found blue green light to be very effective in increasing the rate of photosynthesis of brown and red algae. **In** fact the red algae gave the best result in green light. Since chlorophylls hardly absorb green light it became obvious that some other accessory pigment was involved. Several workers have since then demonstrated the role of phycobilins and carotenoids in photosynthesis. The irradiation of carotenoids causes **fluorescence** of chlorophyll suggesting the transfer of energy by accessory pigments to chlorophyll a during photosynthesis.

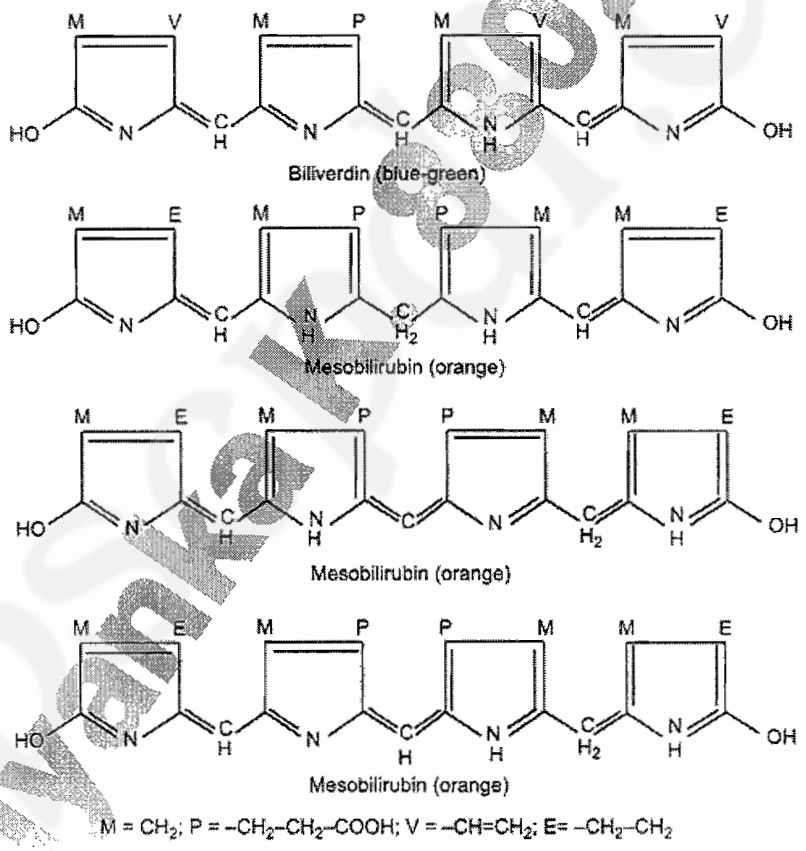


Figure 5: Molecular structure of *phycobilins*

In blue-green and red algae some additional pigments known as phycobilins are present. They are also tetrapyrroles like chlorophylls but the four joined pyrrole rings form a straight chain (Fig. 6). Like anthocyanins they also mask the green colour of the chlorophylls. They are,

however, intimately associated with the chlorophylls. The light absorbed by them can be used in photosynthesis. Phycobilins include red coloured **phycoerythrins** and blue coloured **phycocyanins** found in red and blue-green algae respectively.Phycoerythrin absorbs the green light the best.Phycocyanin absorb the blue light the best.

While chlorophylls and carotenoids are soluble in organic solvent the phycobilins are soluble in hot water. The absorption spectra of phycoerythrin, allophycocyanin and phycocyanin is given:

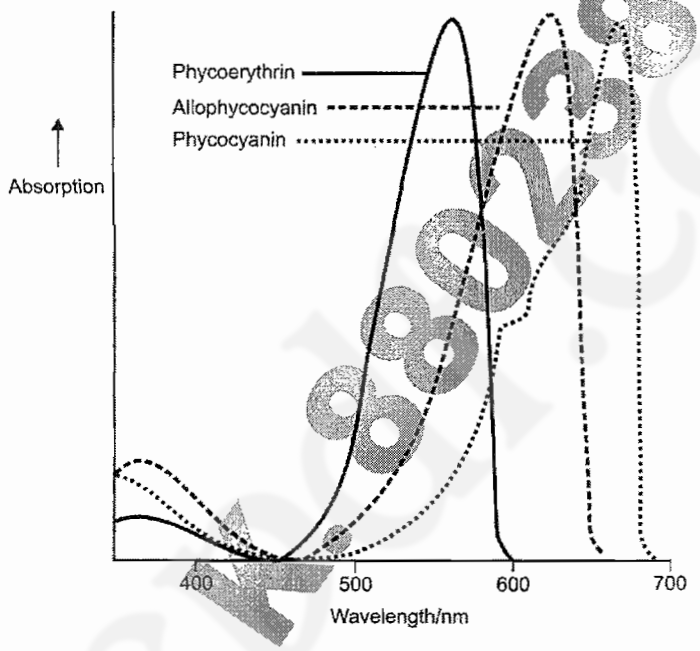


Figure 6: Absorption spectrum of three phycobilins

ANTHOCYANINS

The colour of leaves is modified in certain plants due to the presence of purple pigment called **anthocyanins.** They are formed by several rings of atoms, the rings being joined in complex ways. Anthocyanins are soluble in water, hence they occur in the vacuolar sap of the cells.

This pigment does not take any part in photosynthesis. Anthocyanin is not present in the cytoplasm.

evolution

Reference Study Material

The Light Reactions

THE CONTEXT

Photosynthesis is the physico-chemical process by which plants, algae and photosynthetic bacteria use light energy to drive the synthesis of organic compounds. In plants, algae and certain types of bacteria, the photosynthetic process results in the release of molecular oxygen and the removal of carbon dioxide from the atmosphere that is used to synthesize carbohydrates (oxygenic photosynthesis).

Other types of bacteria use light energy to create organic compounds but do not produce oxygen (anoxygenic photosynthesis). Photosynthesis provides the energy and reduced carbon required for the survival of virtually all life on our planet, as well as the molecular oxygen necessary for the survival of oxygen consuming organisms. In addition, the fossil fuels currently being burned to provide energy for human activity were produced by ancient photosynthetic organisms.

Essentially all free energy utilized by biological systems arises from solar energy that is trapped by the process of photosynthesis. The basic equation of photosynthesis is deceptively simple. Water and carbon dioxide combine to form carbohydrates and molecular oxygen.

$$CO_2 + H_2O \xrightarrow{\text{Light}} (CH_2O) + O_2$$

In this equation, (CH_2O) represents carbohydrate, primarily sucrose and starch. The mechanism of photosynthesis is complex and requires the interplay of many proteins and small molecules. Photosynthesis in green plants takes place in *chloroplasts.*

The energy of light captured by pigment molecules, called chlorophylls, in chloroplasts is used to generate high-energy electrons with great reducing potential. These electrons are used to produce NADPH as well as ATP in a series of reactions called the *light reactions* because they require light. NADPH and ATP formed by the action of light then reduce carbon dioxide and convert it into *3-phosphoglycerate* by a series of reactions called the *Calvin cycle* or the dark reactions.

The amount of energy stored by photosynthesis is enormous. More than 10^{17} kcal (4.2×10^{17} kJ) of free energy is stored annually by photosynthesis on Earth, which corresponds to the assimilation of more than 10^{10} tons of carbon into carbohydrate and other forms of organic matter.

THE LIGHT DEPENDENT STAGE

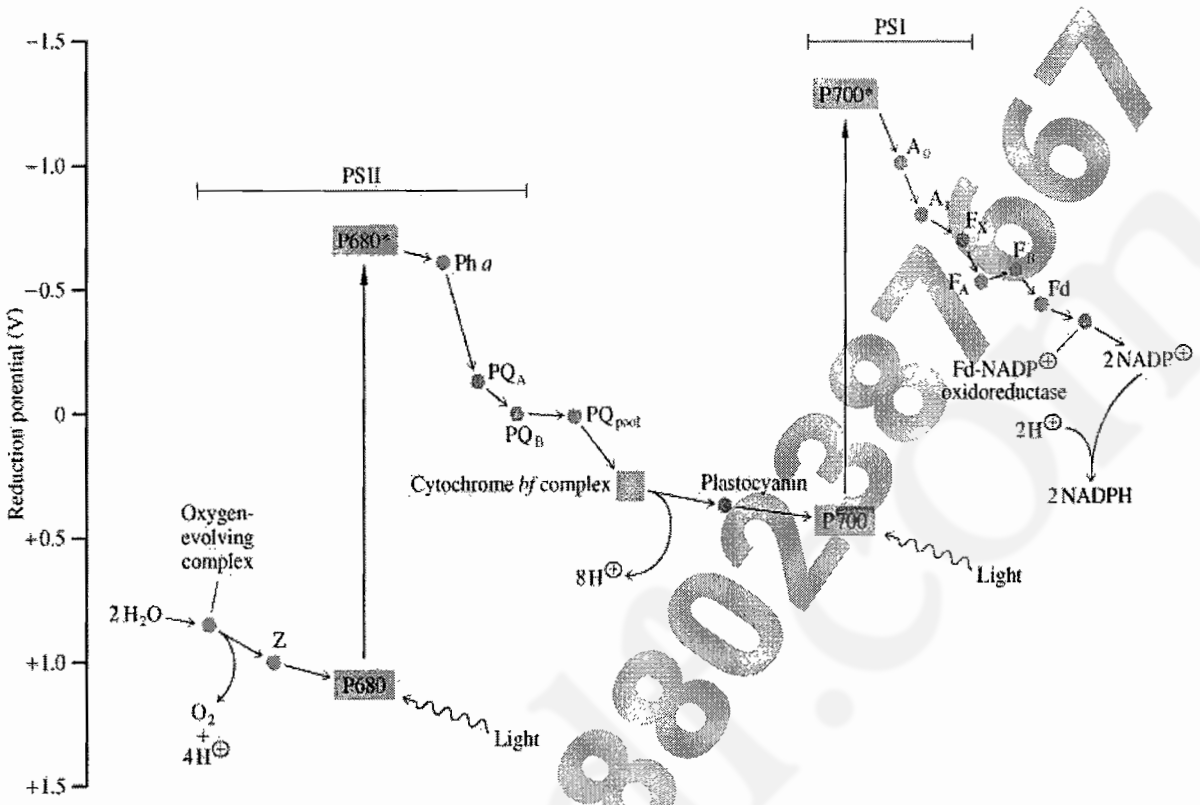
The thylakoid membranes contain the energy-transducing machinery: light-harvesting proteins, reaction centers, electron-transport chains, and ATP synthase. The early events of photosyntheis, the **light dependent stage**, in green plants is mediated by two kinds of membrane-bound, light-sensitive complexes—*photosystem I (PS I)* and *photosystem II (PS II)*.

Photosynthesis by oxygen-evolving organisms depends on the interplay of two photosystems, linked by common intermediates.

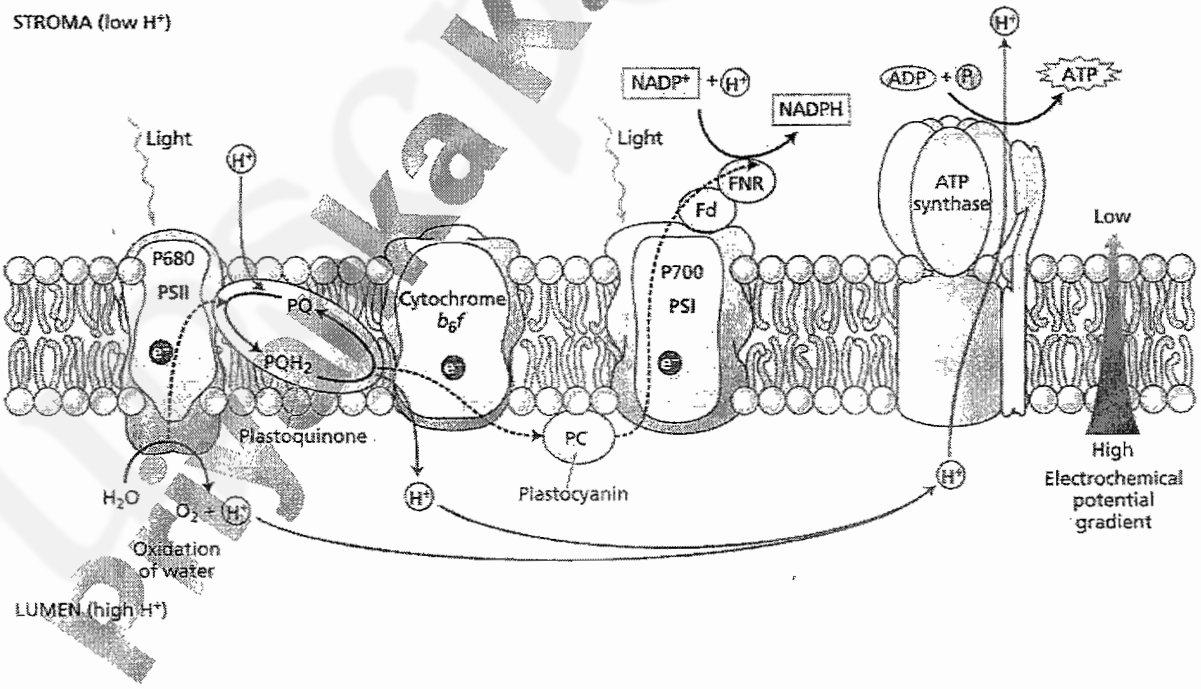
These two systems were discovered because of slight differences in the wavelengths of light to which they respond. Photosystem I responds to light with wavelengths shorter than 700 nm, whereas photosystem II responds to wavelengths shorter than 680 nm. Under normal conditions, electrons flow first through photosystem II, then through cytochrome b_6f, a membrane-bound complex homologous to Q-cytochrome c oxidoreductase from oxidative phosphorylation, and then through photosystem I. The electrons are derived from water: two molecules of H_2O are oxidized to generate a molecule of O_2 for every four electrons sent through this electron-transport chain. The electrons end up reducing $NADP^+$ to NADPH, a versatile reagent for driving biosynthetic processes. These processes generate a proton gradient across the thylakoid membrane that drives the formation of ATP.

NADPH and ATP are together called the **Assimilatory Power** of photosynthesis, as these two compounds are essential to carry out the reduction of CO_2 into Carbohydrates – the metabolic integration of CO_2 or Carbon Assimilation. We can also look upon the light dependent stage of photosynthesis as a preparatory stage for the light independent stage [or, so called **Dark Stage**], where actual CO_2 assimilation into the plant's physiological system takes place.

The scheme of electron flow from the PS II to PS I is called the **Z scheme**, as described by **Hill** and **Bendall**. The Z scheme is called so, because it presents a Zig Zag scheme when we plot the electron transporters along their redox potential values. The Z-scheme is shown below.



The same Z scheme, with the involved complexes of the thylakoid membrane is shown below.



THE STEPS IN DETAIL

The trapping of light energy is the key to photosynthesis. The first event is the absorption of light by a photoreceptor molecule. The principal photoreceptor in the chloroplasts of most green plants is *chlorophyll* a, a substituted tetrapyrrole. The four nitrogen atoms of the pyrroles are coordinated to a magnesium ion. Unlike a porphyrin such as heme, chlorophyll has a reduced pyrrole ring. Another distinctive feature of chlorophyll is the presence of *phytol*, a highly hydrophobic 20-carbon alcohol, esterified to an acid side chain.

Chlorophylls are very effective photoreceptors because they contain networks of alternating single and double bonds. Such compounds are called *polyenes*. They have very strong absorption bands in the visible region of the spectrum, where the solar output reaching Earth also is maximal.

Photo-absorption by PSII

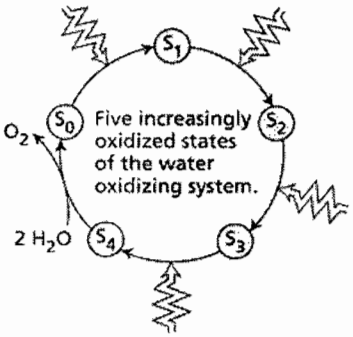
The photochemistry of photosystem II begins with excitation of a special pair of chlorophyll molecules that are bound by the D1 and D2 subunits. This pair of molecules is analogous to the special pair in the bacterial reaction center, but it absorbs light at shorter wavelengths (maximum absorbance at 680 nm) because it consists of chlorophyll *a* molecules rather than bacteriochlorophyll. The special pair is often called *P680*. The energy from the light excites an electron from its ground energy level to an excited energy level.

On excitation, P680 rapidly transfers an electron to a nearby pheophytin (chlorophyll with two H^+ ions in place of the central Mg^{2+} ion). From there, the electron is transferred first to a tightly bound plastoquinone at site Q_A and then to an exchangeable plastoquinone at site Q_B. With the arrival of a second electron and the uptake of two protons, the exchangeable plastoquinone is reduced to QH_2 and becomes mobile.

Photolysis of Water and Release of O_2

$P680^+$, a very strong oxidant, extracts electrons from water molecules bound at the manganese center. The structure of this center, which includes four manganese ions, a calcium ion, a chloride ion, and a tyrosine residue that forms a radical, has not been fully established.

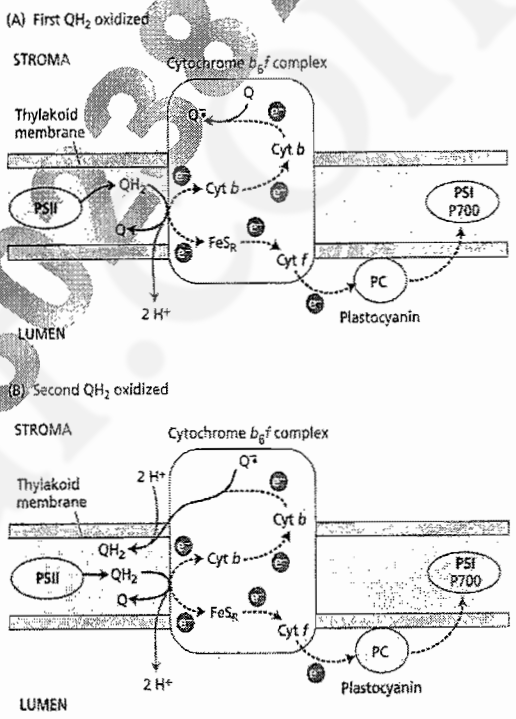
Manganese can exist in multiple oxidation states (Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{5+}) and to form strong bonds with oxygen-containing species. The manganese center, in it reduced form, oxidizes two molecules of water to form a single molecule of oxygen. Each time the absorbance of a photon kicks an electron out of P680, the positively charged special pair extracts an electron from the manganese center.



Thus, four photochemical steps are required to extract the electrons and reduce the manganese center. The four electrons harvested from water are used to reduce two molecules of Q to QH_2.

Photosystem II spans the thylakoid membrane such that the site of quinone reduction is on the side of the stroma, whereas the manganese center and, hence, the site of water oxidation lies in the thylakoid lumen. Thus, the two protons that are taken up with the reduction of each molecule of plastoquinone come from the stroma, and the four protons that are liberated in the course of water oxidation are released into the lumen. This distribution of protons generates a proton gradient across the thylakoid membrane characterized by an excess of protons in the thylakoid lumen compared with the stroma.

Proton-Gradient Direction. Photosystem II releases protons into the thylakoid lumen and takes them up from the stroma. The result is a pH gradient across the thylakoid membrane with an excess of protons (low pH) inside.



It is important to be noted that the four manganese ions act as charge accumulator for photolysis of water and extraction of electrons.

This mode has been described on the basis of a Clock Model, according to which, in the cluster of four manganese ions – there are different oxidation steps. Water is photolysesd only when this cluster of four manganese ions attains its maximum oxidation state.

The Reactions at the Cyt b_6f Complex

The plastoquinol (QH_2)- a mobile electron carrier- produced by photosystem II contributes its electrons to continue the electron chain. These electrons are transferred to the ***cytochrome* b_6f complex**. The cytochrome b_6f complex includes four subunits: a 23-kd cytochrome with two *b*-type hemes, a 20-kd *Rieske-type* Fe-S protein, a 33-kd cytochrome *f* with a *c*-type cytochrome, and a 17-kd chain. This complex catalyzes the reaction through the **Q cycle**.

In the first half of the Q cycle, plastoquinol is oxidized to plastoquinone, one electron at a time. The electrons from plastoquinol flow through the Fe-S protein to convert oxidized plastocyanin into its reduced form. The oxidation of plastoquinol results in the release of two protons into the thylakoid lumen. In the second half of the Q cycle, cytochrome *bf* reduces a second molecule of plastoquinone from the Q pool to plastoquinol, taking up two protons from one side of the membrane, and then reoxidizes plastoquinol to release these protons on the other side. The enzyme is oriented so that protons are released into the thylakoid lumen and taken up from the stroma, contributing further to the proton gradient across the thylakoid membrane.

Transfer of the Electrons to the PS I

Plastocyanin is a **mobile** electron carrier that links cytochrome *bf* complex to the PS I. It is a small, soluble protein **with a single copper ion** bound by a cysteine residue, two histidine residues, and a methionine residue in a distorted tetrahedral arrangement. This geometry facilitates the interconversion between the Cu^{2+} and the Cu^{+} states and sets the reduction potential at an appropriate value relative to that of plastoquinol. Plastocyanin is intensely blue in color in its oxidized form, marking it as a member of the "blue copper protein," or type I copper protein family.

Reactions at the PS I

The final stage of the light reactions is catalyzed by photosystem I. The core of this system is a pair of similar subunits psaA (83 kd) and psaB (82 kd).

A special pair of chlorophyll *a* molecules lies at the center of the structure and absorb light maximally at 700 nm. This **center, *P700*, initiates photoinduced charge separation at PS I.**

The electron is transferred down a pathway through chlorophyll at site A_0 and quinone at site A_1 to a set of 4Fe-4S clusters. From there, the electron is transferred to ferredoxin (Fd), a soluble protein containing a 2Fe-2S cluster coordinated to four cysteine residues. The positive charge of $P700^+$ is neutralized by the transfer of an electron from reduced plastocyanin.

Light absorption induces electron transfer from P700 down an electron-transfer pathway that includes a chlorophyll molecule, a quinone molecule, and three 4Fe-4S clusters to reach ferredoxin. The positive charge left on P700 is neutralized by electron transfer from reduced plastocyanin.

Although reduced ferredoxin is a strong reductant, it is not useful for driving many reactions, in part because ferredoxin carries only one available electron. In contrast, NADPH, a two-electron reductant, is widely used in biosynthetic processes, including the reactions of the Calvin cycle.

The reduction of $NADP^+$ is carried out by *ferredoxin-$NADP^+$reductase* [FNR] a flavoprotein. The bound FAD moiety in this enzyme accepts electrons, one at a time, from two molecules of reduced ferredoxin as it proceeds from its oxidized form, through a semiquinone intermediate, to its fully reduced form. The enzyme then transfers a hydride ion to $NADP^+$ to form NADPH.

This reaction takes place on the stromal side of the membrane. Hence, the uptake of a proton in the reduction of $NADP^+$ further contributes to the proton gradient across the thylakoid membrane.

Thus, the cooperation between photosystem I and photosystem II creates electron flow from H_2O to NADPH. The pathway of electron flow is called the *Z scheme of photosynthesis* because the redox diagram from P680 to P700* looks like the letter Z.

ATP SYNTHESIS OR PHOTOPHOPHORYLATION

In 1966, André Jagendorf showed that chloroplasts synthesize ATP in the dark when an artificial pH gradient is imposed across the thylakoid membrane. To create this transient pH gradient, he soaked chloroplasts in a pH 4 buffer for several hours and then rapidly mixed them with a pH 8 buffer containing ADP and P_i. The pH of the stroma suddenly increased to 8, whereas the pH of the thylakoid space remained at 4. *A burst of ATP synthesis then accompanied the disappearance of the pH gradient across the thylakoid membrane.* This incisive experiment was one of the first to unequivocally support the hypothesis put forth by Peter Mitchell that ATP synthesis is driven by proton-motive force.

The principles by which ATP synthesis takes place in chloroplasts are nearly identical with those for oxidative phosphorylation.

Light induces electron transfer through photosystems II and I and the cytochrome *bf* complex. At various stages in this process, protons are released into the thylakoid lumen or taken up from the stroma, generating a proton gradient. Such a gradient can be maintained because the thylakoid membrane is essentially impermeable to protons. *The thylakoid space becomes markedly acidic, with the pH approaching 4. The light-induced transmembrane proton gradient is about 3.5 pH units.*

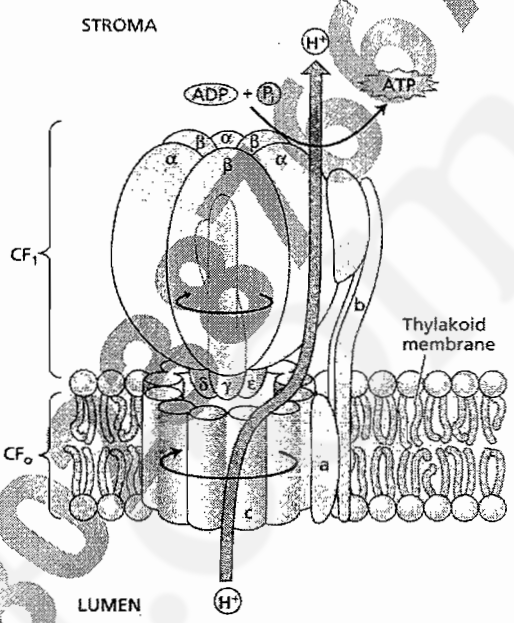
Energy inherent in the proton gradient, called the *proton-motive force (Δp)*, is described as the sum of two components: a charge gradient and a chemical gradient. In chloroplasts, nearly all of Δp arises from the pH gradient, whereas, in mitochondria, the contribution from the membrane potential is larger. The reason for this difference is that the thylakoid membrane is quite permeable to Cl^- and Mg^{2+}. The light-induced transfer of H^+ into the thylakoid space is accompanied by the transfer of either Cl^- in the same direction or Mg^{2+} (1 Mg^{2+} per 2 H^+) in the opposite direction. Consequently, electrical neutrality is maintained and no membrane potential is generated. A pH gradient of 3.5 units across the thylakoid membrane corresponds to a proton-motive force of 0.20 V or a ΔG of -4.8 kcal mol^{-1} (-20.0 kJ mol^{-1}).

The proton-motive force generated by the light reactions is converted into ATP by the *ATP synthase* of chloroplasts, also called the *CF_1-CF_0complex* (*C* stands for chloroplast and *F* for factor). CF_1-CF_0 ATP synthase closely resembles the F_1-F_0 complex of mitochondria.

CF_0 conducts protons across the thylakoid membrane, whereas CF_1 catalyzes the formation of ATP from ADP and P_i.

CF_0 is embedded in the thylakoid membrane. It consists of four different polypeptide chains known as I (17 kd), II (16.5 kd), III (8 kd), and IV (27 kd) having an estimated stoichiometry of 1:2:12:1. Subunits I, II, and III correspond to subunits **a, b,** and **c,** respectively, of the mitochondrial F_0 subunit, and subunit IV is similar in sequence to subunit **a.** CF_1, the site of ATP synthesis, has a subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$. The β subunits contain the catalytic sites, similar to the F_1 subunit of mitochondrial ATP synthase.

Protons flow *out* of the thylakoid lumen through ATP synthase into the stroma. Because CF_1 is on the stromal surface of the thylakoid membrane, the newly synthesized ATP is released directly into the stromal space. Recall that NADPH formed through the action of photosystem I and ferredoxin-$NADP^+$ reductase also is released into the stromal space. Thus, *ATP and NADPH, the products of the light reactions of photosynthesis, are appropriately positioned for the subsequent dark reactions, in which CO_2 is converted into carbohydrate.*



CYCLIC ELECTRON FLOW

An alternative pathway for electrons arising from P700, the reaction center of photosystem I, contributes to the versatility of photosynthesis. The electron in reduced ferredoxin can be transferred to the cytochrome *bf* complex rather than to $NADP^+$. This electron then flows back through the cytochrome *bf* complex to reduce plastocyanin, which can then be reoxidized by $P700^+$ to complete a cycle. The net outcome of this cyclic flow of electrons is the pumping of protons by the cytochrome *bf* complex. The resulting proton gradient then drives the synthesis of ATP. In this process, called *cyclic photophosphorylation, ATP is generated without the concomitant formation of NADPH.* Photosystem II does not participate in cyclic photophosphorylation, and so O_2 is not formed from H_2O. Cyclic photophosphorylation takes place when $NADP^+$ is unavailable to accept electrons from reduced ferredoxin, because of a very high ratio of NADPH to $NADP^+$.

evolution
Reference Study Material

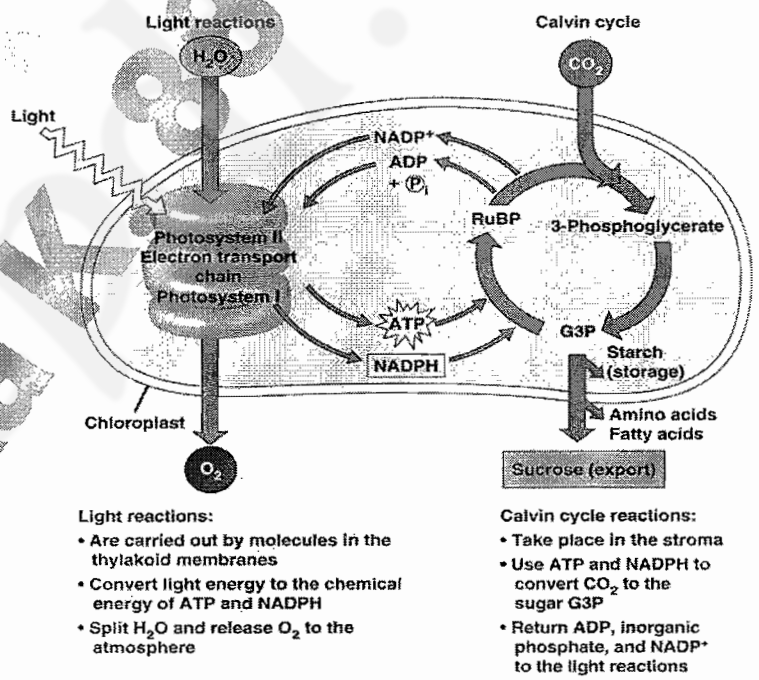
The Dark Reactions of Photosynthesis

THE CALVIN CYCLE

The **Calvin cycle** — a *universal part of photosynthesis* — (or **Calvin-Benson cycle** or carbon fixation) is a series of biochemical reactions that takes place in the stroma of chloroplasts in photosynthetic organisms. It reduces carbon atoms in carbon dioxide to the reduced state as a carbohydrate. It was discovered by Melvin Calvin, James Bassham and Andrew Benson at the University of California, Berkeley.

Calvin Cycle in the Overall Context of Photosynthesis

Photosynthesis proceeds in two parts: the light dependent reactions and the light independent reactions.



The light dependent reactions transform light energy into ATP and biosynthetic reducing power, NADPH. *NADPH is the currency of readily available reducing power in cells*. The phosphoryl group on the 2'-hydroxyl group of one of the ribose units of NADPH distinguishes NADPH from NADH.

The light independent reactions, which constitute the **Calvin cycle**, reduce carbon atoms in carbon dioxide to the reduced state as a carbohydrate, using ATP and biosynthetic reducing power, NADPH — generated during the light dependent stage. The components of the Calvin cycle are also called the dark reactions because, in contrast with the light dependent reactions, these reactions do not directly depend on the presence of light.

The relation between the Calvin Cycle and the light dependent stage of photosynthesis is shown in the figure above.

The Calvin Cycle Reactions

The source of the carbon atoms in the Calvin cycle is the simple molecule carbon dioxide.

The Calvin cycle comprises three stages

1. The fixation of CO_2 by ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate.
2. The reduction of 3-phosphoglycerate to form hexose sugars.
3. The regeneration of ribulose 1,5-bisphosphate so that more CO_2 can be fixed.

The sum of reactions in the Calvin cycle is the following:

$$3\ CO_2 + 6\ NADPH + 6\ H^+ + 9\ ATP \rightarrow C_3(H_2O)_3 + 3\ H_2O + 6\ NADP^+ + 9\ ADP$$

Steps of the Calvin cycle

Reaction 1. The first step in the Calvin cycle is the fixation of CO_2. The CO_2 molecule condenses with ribulose 1,5-bisphosphate to form an unstable six-carbon compound, which is rapidly hydrolyzed to two molecules of 3-phosphoglycerate. The enzyme RUBISCO catalyses the carboxylation of Ribulose-1,5-bisphosphate, a 5 carbon compound, by carbon dioxide (a total of 6 carbons). Two molecules of 3-phosphoglycerate, a 3-carbon compound, are created. (also called:, glycerate 3-phosphate, 3-phosphoglyceric acid, 3PGA).

More on RUBISCO: The initial incorporation of CO_2 into 3-phosphoglycerate was revealed through the use of a carbon-14 radioactive tracer. This highly exergonic reaction is catalyzed by ***ribulose 1,5-bisphosphate carboxylase/oxygenase*** (usually called RUBISCO), an enzyme located on the stromal surface of the thylakoid membranes of chloroplasts. **This important reaction is the rate-limiting step in hexose synthesis.**

RUBISCO in chloroplasts consists of eight large (L, 55-kd) subunits and eight small (S, 13-kd) ones. Hence it is also called a **dimer of octamers**. Each L chain contains a catalytic site and a regulatory site. It is encoded by the plastidial genome itself. The S chains (encoded by the nucleus located genes, which express under the control of Phytochrome) enhance the catalytic activity of the L chains. This enzyme is very abundant in chloroplasts, constituting more than 16% of their total protein. In fact, RUBISCO is the most abundant protein enzyme and probably the most abundant protein in the biosphere. Large amounts are present because RUBISCO is a slow enzyme; its maximal catalytic rate is only 3 s^{-1}.

RUBISCO requires a bound divalent metal ion for activity, usually *magnesium ion*. This metal ion serves to activate a bound substrate molecule by stabilizing a negative charge. Interestingly, a CO_2 molecule other than the substrate is required to complete the assembly of the Mg^{2+} binding site in RUBISCO. This CO_2 molecule adds to the uncharged α-amino group of lysine 201 to form a *carbamate*. This negatively charged adduct then binds the Mg^{2+} ion. The formation of the carbamate is facilitated by the enzyme *RUBISCO activase*, although it will also form spontaneously at a lower rate.

The metal center plays a key role in binding ribulose 1,5-bisphosphate and activating it so that it will react with CO_2. Ribulose 1,5-bisphosphate binds to Mg^{2+} through its keto group and an adjacent hydroxyl group. This complex is readily deprotonated to form an enediolate intermediate. This reactive species couples with CO_2, forming the new carbon-carbon bond. The resulting product is coordinated to the Mg^{2+} ion through three groups, including the

newly formed carboxylate. A molecule of H_2O is then added to this α-ketoacid to form an intermediate that cleaves to form two molecules of 3-phosphoglycerate.

Catalytic Imperfection: RUBISCO Also Catalyzes a Wasteful Oxygenase Reaction: The reactive intermediate generated on the Mg^{2+} ion sometimes reacts with O_2 instead of CO_2. Thus, RUBISCO also catalyzes a deleterious oxygenase reaction. The products of this reaction are *phosphoglycolate* and *3-phosphoglycerate*. The phosphoglycolate can be salvaged and used for biosynthetic reactions but the pathway for achieving this releases CO_2 and NH_4^+ and wastes metabolic energy. Because the net result of this process is to consume O_2 and release CO_2, it is known as **photorespiration**.

The rate of the carboxylase reaction is four times that of the oxygenase reaction under normal atmospheric conditions at 25°C; the stromal concentration of CO_2 is then 10 μM and that of O_2 is 250 μM. The oxygenase reaction, like the carboxylase reaction, requires that lysine 201 be in the carbamate form. Because this carbamate forms only in the presence of CO_2, this property would prevent RUBISCO from catalyzing the oxygenase reaction exclusively when CO_2 is absent. However, this is a major problem for plants in hot climates. **1.** The plants close the stomata in their leaves to conserve water but this leads to a drop in the CO_2 concentration within the leaf, favoring photorespiration. **2.** In addition, as temperature rises, the oxygenase activity of RUBISCO (using O_2) increases more rapidly than the carboxylase activity (using CO_2), again favoring photorespiration. **3.** Moreover, at high temperature, the dissolubility of CO_2 in water falls sharply.

To avoid these problems, some plants adapted to live in hot climates, such as maize and sugar cane, have evolved a mechanism to maximize the carboxylase activity of RUBISCO. In these plants, carbon fixation using the Calvin Cycle takes place only in bundle-sheath cells that are protected from the air by mesophyll cells. Since the bundle-sheath cells are not exposed to air, the O_2 concentration is low. The CO_2 is transported from the air via the mesophyll cells to the bundle-sheath cells by combining with three-carbon molecules (C_3) to produce four-carbon molecules (C_4). These enter the bundle-sheath cells where they are broken down to C_3 compounds, releasing CO_2.The C_3 molecules return to the mesophyll cell to accept more CO_2. This cycle ensures a high CO_2 concentration for the carboxylase activity of RUBISCO action in the bundle-sheath cells.

Since it relies on CO_2 transport via four-carbon molecules, it is called the C4 pathway and plants that use this mechanism are called C4 plants. All other plants are called C3 plants since they trap CO_2 directly as the three-carbon compound 3-phosphoglycerate.

Reaction 2. The enzyme phosphoglycerate kinase catalyses the phosphorylation of 3PGA by ATP (which was produced in the light-dependent stage). 1,3-bisphosphoglycerate (or 1,3 BPG, glycerate-1,3-bisphosphate) and ADP are the products. Since, two PGAs are produced for every CO_2 that enters the cycle, so this step happens twice.

Reaction 3. The enzyme G3P dehydrogenase catalyses the reduction of 1,3BPG by NADPH (which was another product of the light-dependent stage). Glyceraldehyde 3-phosphate (also G3P, GP) is produced, and the NADPH itself was oxidised and hence becomes $NADP^+$.

Reaction 4. Triose phosphate isomerase converts some G3P reversibly into dihydroxyacetone phosphate (DHAP), also a 3-carbon molecule.

Reaction 5. Aldolase and Fructose-1,6-bisphosphatase convert some of these two into fructose-6-phosphate (6C). A phosphate ion is lost to ADP.

Up to this point, as per the overall equation given above, 3 carbon dioxide molecules would have been converted, with the use of 3 RuBP, 6 ATP and 6 NADPH, to 6 G3P molecules. One G3P then exits the cycle, while 5 of these G3P molecules continue in the cycle, meant to regenerate the 3 molecule of RuBP. The regeneration steps include:

Reaction 6. F6P is then combined with another G3P (total 9C) and then cleaved into xylulose-5-phosphate (X5P) and erythrose-4-phosphate by transketolase.

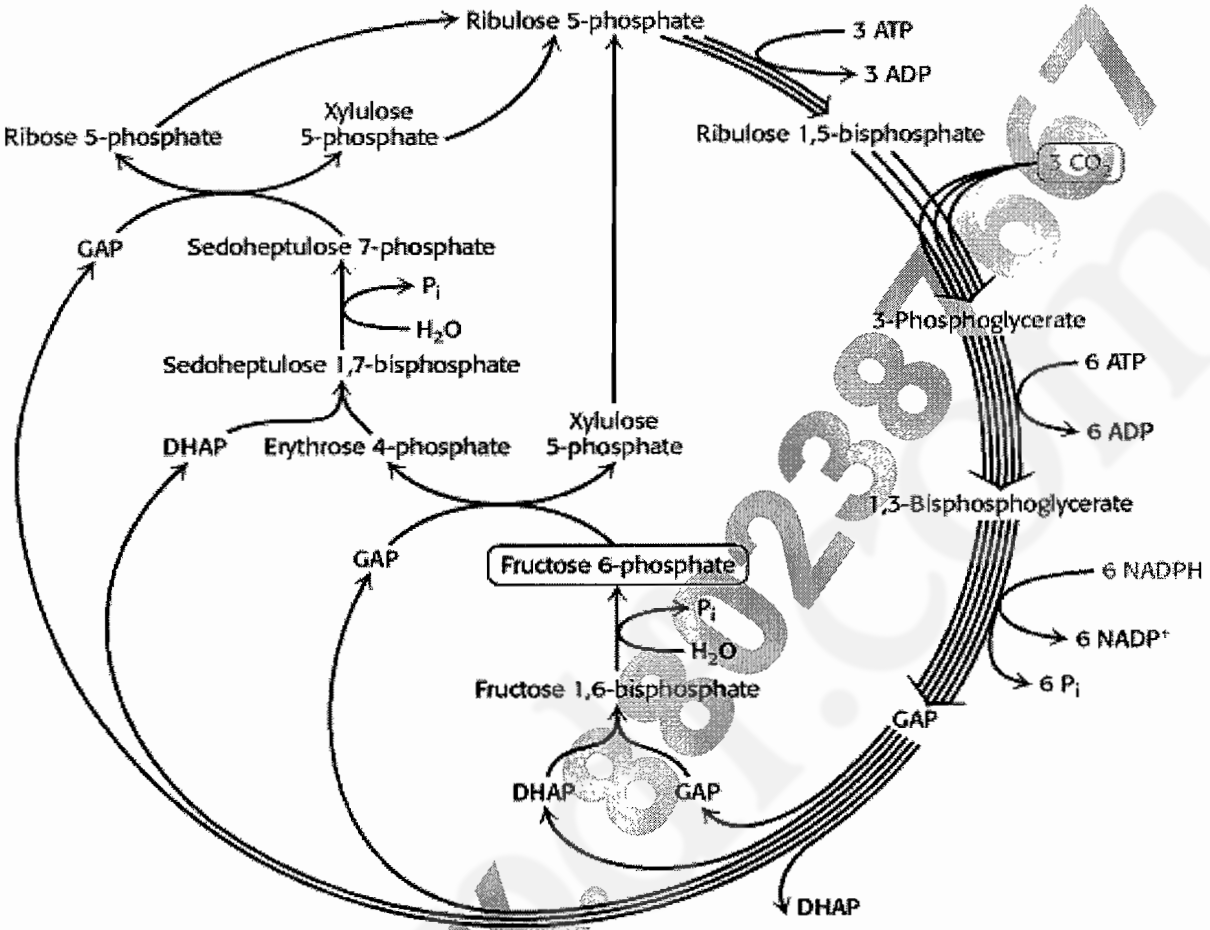
Reaction 7. E4P and DHAP are converted into sedoheptulose-7-phosphate (7C) by S1,7BPase. A phosphate ion is lost to ADP.

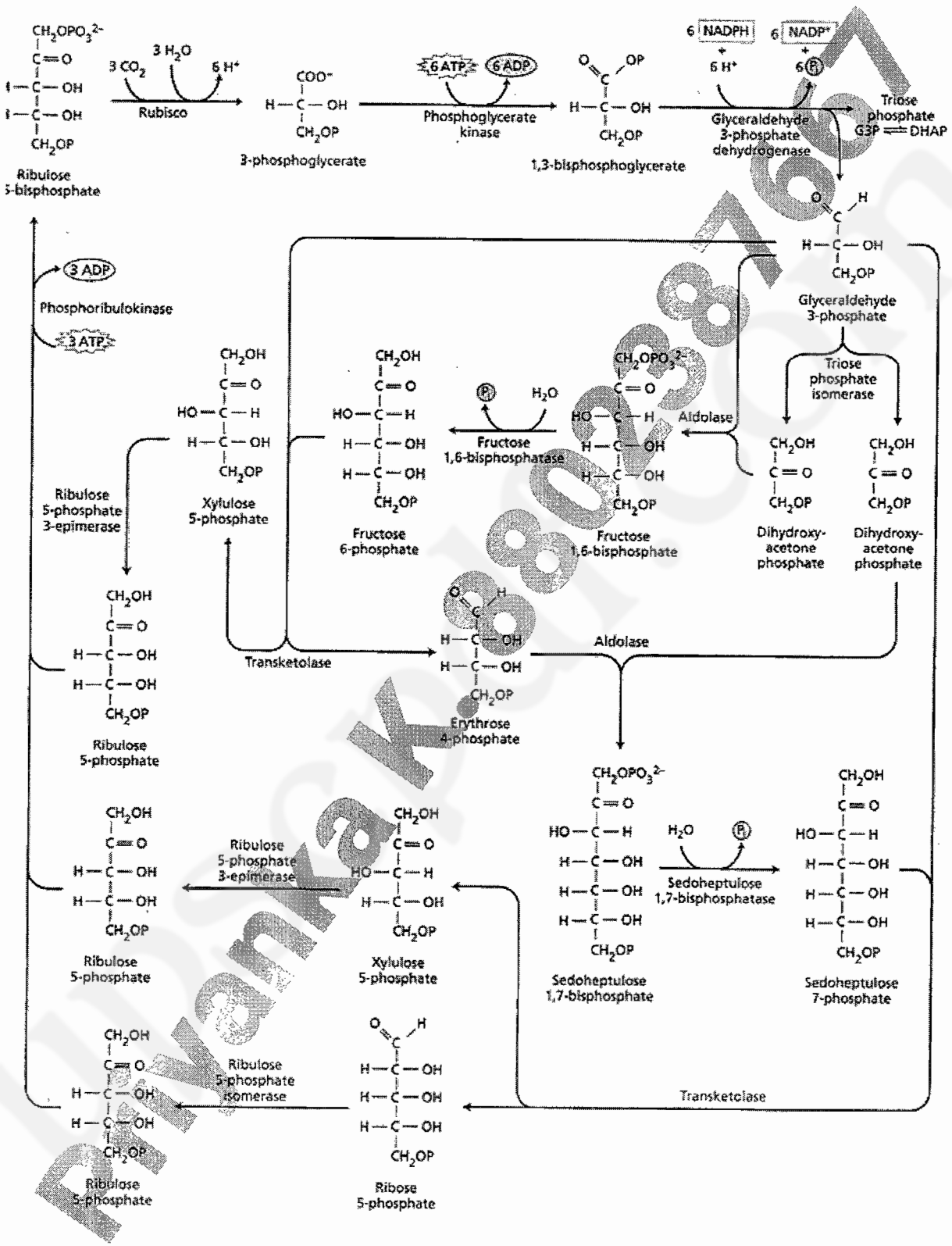
Reaction 8. S7P is then combined with another G3P (total 10C) and then cleaved into another X5P and ribose-5-phosphate (R5P) again by transketolase.

Reaction 9. X5P is converted into ribulose-5-phosphate (Ru5P, RuP) by epimerase. R5P is also converted into RuP by ribose isomerase.

Reaction 10. Finally, phosphoribulokinase phosphorylates RuP into RuBP, ribulose-1,5-bisphosphate, completing the Calvin *cycle*. This requires the input of one ATP.

The five G3P retained in the cycle earlier is converted into 3 molecules of RuBP (5C), so 5 G3Ps (15C) were needed to produce 3 RuBPs (15C). 3 ATPs were also needed in the last step, giving a total of 9 ATPs used up per 3 CO_2s.





Energetics of the Calvin Cycle

Six rounds of the Calvin cycle are required, because one carbon atom is reduced in each round. Twelve molecules of ATP are expended in phosphorylating 12 molecules of 3-phosphoglycerate to 1,3-bisphosphoglycerate, and 12 molecules of NADPH are consumed in reducing 12 molecules of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate. An additional six molecules of ATP are spent in regenerating ribulose 1,5-bisphosphate. If we look from the stand point of a single hexose molecule synthesis, a balanced equation for the net reaction of the Calvin cycle is:

$$6\,CO_2 + 18\,ATP + 12\,NADPH + 12\,H_2O \longrightarrow C_6H_{12}O_6 + 18\,ADP + 18\,P_i + 12\,NADP^+ + 6\,H^+$$

REGULATION OF CALVIN CYCLE

The light reactions lead to changes in the stroma — namely, an increase in pH and in Mg^{2+}, NADPH, and reduced ferredoxin concentration-all of which contribute to the activation of certain Calvin cycle enzymes. The major regulatory pathways of the regulation of the Calvin cycle are described below.

The rate-limiting step in the Calvin cycle is the carboxylation of ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate. *The activity of RUBISCO increases markedly on illumination.*

The addition of CO_2 to lysine 201 of RUBISCO to form the carbamate is essential for Mg^{2+} coordination and, hence, catalytic activity. Carbamate formation is favored by alkaline pH and high concentrations of Mg^{2+} ion in the stroma, both of which are consequences of the light-driven pumping of protons from the stroma into the thylakoid space. Magnesium ion concentration rises because Mg^{2+} ions from the thylakoid space are released into the stroma to compensate for the influx of protons.

Light-driven reactions lead to electron transfer from water to ferredoxin and, eventually, to NADPH. Both reduced ferredoxin and NADPH regulate enzymes from the Calvin cycle. One key protein in these regulatory processes is *thioredoxin*, a 12-kd protein containing neighboring cysteine residues that cycle between a reduced sulfhydryl and an oxidized disulfide form. The reduced form of thioredoxin activates many biosynthetic enzymes by reducing disulfide bridges that control their activity and inhibits several degradative enzymes by the same means. Such enzymes include, Fructose 1,6-bisphosphatase, Glyceraldehyde 3-phosphate dehydrogenase, Sedoheptulose bisphosphatase, Ribulose 5′-phosphate kinase and $NADP^+$-malate dehydrogenase. In chloroplasts, oxidized thioredoxin is reduced by ferredoxin in a reaction catalyzed by *ferredoxin-thioredoxin reductase.* Thus, *the activities of the light and dark reactions of photosynthesis are coordinated through electron transfer from reduced*

ferredoxin to thioredoxin and then to component enzymes containing regulatory disulfide bonds.

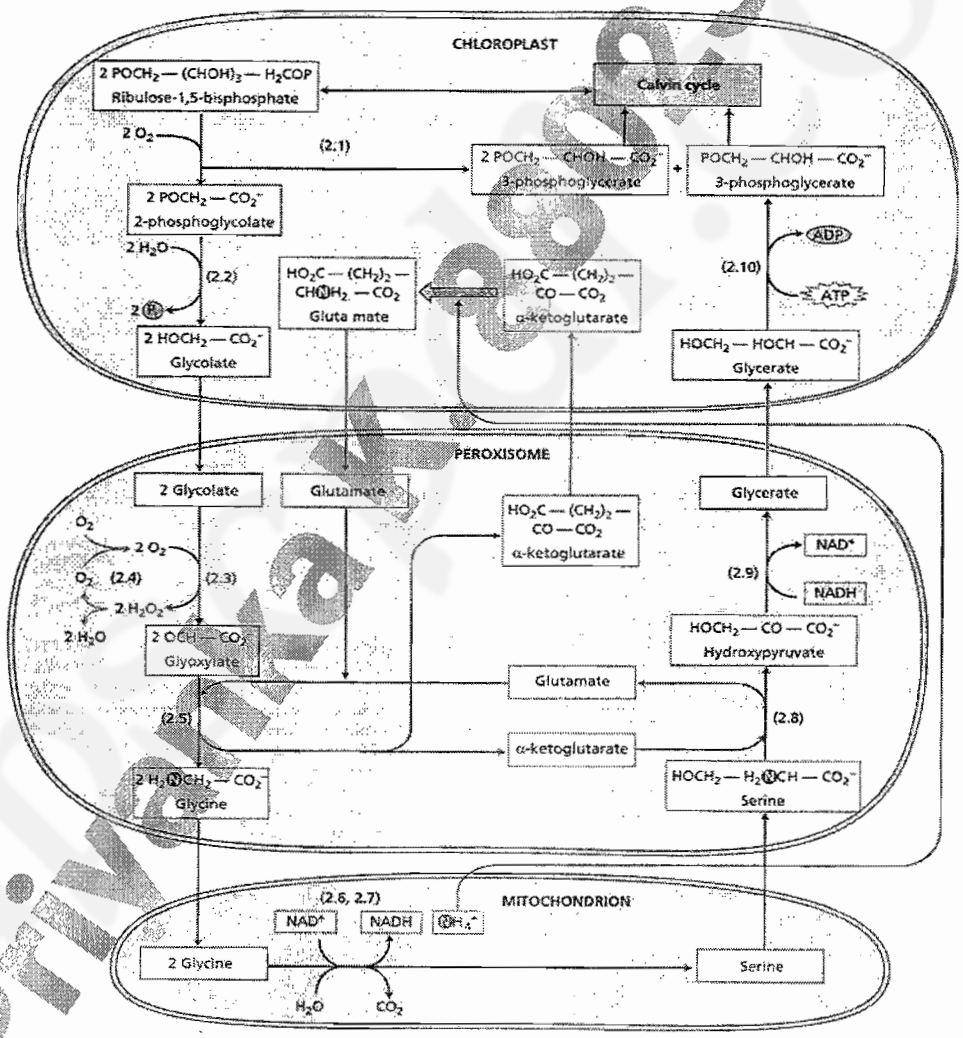
Other means of control also exist.

For instance, phosphoribulose kinase and glyceraldehyde 3-phosphate dehydrogenase also are regulated by NADPH directly. In the dark, these enzymes associate with a small protein called CP12 to form a large complex in which the enzymes are inactivated. NADPH generated in the light reactions binds to this complex, leading to the release of the enzymes. Thus, the activity of these enzymes depends first on reduction by thioredoxin and then on the NADPH-mediated release from CP12.

Photorespiration

An important property of RUBISCO is its ability to catalyze both the carboxylation and the oxygenation of RuBP. Oxygenation is the primary reaction in a process known as photorespiration. Because photosynthesis and photorespiration work in diametrically opposite directions, photorespiration results in loss of CO_2 from cells that are simultaneously fixing CO_2 by the Calvin cycle (Ogren 1984; Leegood et al. 1995).

The C_2 oxidative photosynthetic carbon cycle includes the reactions that result in the partial recovery of carbon lost through oxidation. The steps are as follows:



Enzyme	Reaction
1. Ribulose-1,5-bisphosphate carboxylase/oxygenase (chloroplast)	2 Ribulose-1,5-bisphosphate + 2 O_2 → 2 phosphoglycolate + 2 3-phosphoglycerate + 4 H^+
2. Phosphoglycolate phosphatase (chloroplast)	2 Phosphoglycolate + 2 H_2O → 2 glycolate + 2 P_i
3. Glycolate oxidase (peroxisome)	2 Glycolate + 2 O_2 → 2 glyoxylate + 2 H_2O_2
4. Catalase (peroxisome)	2 H_2O_2 → 2 H_2O + O_2
5. Glyoxylate:glutamate aminotransferase (peroxisome)	2 Glyoxylate + 2 glutamate → 2 glycine + 2 α-ketoglutarate
6. Glycine decarboxylase (mitochondrion)	Glycine + NAD^+ + H^+ + H_4-folate → NADH + CO_2 + NH_4^+ + methylene-H_4-folate
7. Serine hydroxymethyltransferase (mitochondrion)	Methylene-H_4-folate + H_2O + glycine → serine + H_4-folate
8. Serine aminotransferase (peroxisome)	Serine + α-ketoglutarate → hydroxypyruvate + glutamate
9. Hydroxypyruvate reductase (peroxisome)	Hydroxypyruvate + NADH + H^+ → glycerate + NAD^+
10. Glycerate kinase (chloroplast)	Glycerate + ATP → 3-phosphoglycerate + ADP + H^+

Note: Upon the release of glycolate from the chloroplast (reactions 2 → 3), the interplay of this organelle with the peroxisome and the mitochondrion drives the following overall reaction:

2 Glycolate + glutamate + O_2 → glycerate + α-ketoglutarate + NH_4^+ + CO_2 + H_2O

The 3-phosphoglycerate formed in the chloroplast (reaction 10) is converted to ribulose-1,5-bisphosphate via the reductive and regenerative reactions of the Calvin cycle. The ammonia and α-ketoglutarate are converted to glutamate in the chloroplast by ferrodoxin-linked glutamate synthase (GOGAT).

P_i stands for inorganic phosphate.

The incorporation of one molecule of O_2 into ribulose-1,5-bisphosphate generates an unstable intermediate that rapidly splits into 2-phosphoglycolate and 3-phosphoglycerate. The ability to catalyze the oxygenation of ribulose-1,5-bisphosphate is a property of all RUBISCOs, regardless of taxonomic origin. Even the RUBISCO from anaerobic, autotrophic bacteria catalyzes the oxygenase reaction when exposed to oxygen.

As alternative substrates for RUBISCO, CO_2 and O_2 compete for reaction with ribulose-1,5-bisphosphate because carboxylation and oxygenation occur within the same active site of the enzyme. Offered equal concentrations of CO_2 and O_2 in a test tube, angiosperm RUBISCOs fix CO_2 about 80 times faster than they oxygenate. However, an aqueous solution in equilibrium with air at 25°C has a CO_2:O_2 ratio of 0.0416. At these concentrations, carboxylation in air outruns oxygenation by a ratio of mere three to one.

The C_2 oxidative photosynthetic carbon cycle acts as a **scavenger operation** to recover fixed carbon lost during photorespiration by the oxygenase reaction of RUBISCO. The 2-phosphoglycolate formed in the chloroplast by oxygenation of ribulose-1,5-bisphosphate is rapidly hydrolyzed to glycolate by a specific chloroplast phosphatase.

Subsequent metabolism of the glycolate involves the cooperation of two other organelles: peroxisomes and mitochondria.

Glycolate leaves the chloroplast via a specific transporter protein in the envelope membrane and diffuses to the peroxisome. There it is oxidized to glyoxylate and hydrogen peroxide (H_2O_2) by a flavin mononucleotide-dependent oxidase: glycolate oxidase. The vast amounts of hydrogen peroxide released in the peroxisome are destroyed by the action of catalase while

the glyoxylate undergoes transamination. The amino donor for this transamination is probably glutamate, and the product is the amino acid glycine.

Glycine leaves the peroxisome and enters the mitochondrion. There the glycine decarboxylase multienzyme complex catalyzes the conversion of two molecules of glycine and one of NAD+ to one molecule each of serine, NADH, NH_4 + and CO_2.This multienzyme complex, present in large concentrations in the matrix of plant mitochondria, comprises four proteins, named H-protein (a lipoamide-containing polypeptide), P-protein (a 200 kDa, homodirner, pyridoxal phosphate-containing protein), T-protein (a folate-dependent protein), and L-protein (a flavin adenine nucleotide-containing protein).

The ammonia formed in the oxidation of glycine diffuses rapidly from the matrix of mitochondria to chloroplasts, where glutamine synthetase combines it with carbon skeletons to form amino acids. The newly formed serine leaves the mitochondria and enters the peroxisome, where it is converted first by transamination to and then by an NADHdependent reduction to glycerate.

A malate-oxaloacetate shuttle transfers NADH from the cytoplasm into the peroxisome, thus maintaining an adequate concentration of NADH for this reaction. Finally, glycerate reenters the chloroplast, where it is phosphorylated to yield 3-phosphoglycerate.

In photorespiration, various compounds are circulated in concert through two cycles. In one of the cycles, carbon exits the chloroplast in two molecules of glycolate and returns in one molecule of glycerate. In the other cycle, nitrogen exits the chloroplast in one molecule of glutamate and returns in one molecule of ammonia (together with one molecule of a-ketoglutarate).

Thus overall, two molecules of phosphoglycolate (four carbon atoms), lost from the Calvin cycle by the oxygenation of RuBP, are converted into one molecule of 3-phosphoglycerate (three carbon atoms) and one CO_2 In other words, 75% of the carbon lost by the oxygenation of ribulose-l,5-bisphosphate is recovered by the PCO carbon cycle and returned to the Calvin cycle (Lorimer 1981).

On the other hand, the total organic nitrogen remains unchanged because the formation of inorganic nitrogen (NH_4^+) in the mitochondrion is balanced by the synthesis of glutamine in the chloroplast. Similarly, the use of NADH in the peroxisome (by hydroxypyruvate reductase) is balanced by the reduction of NAD^+ in the mitochondrion (by glycine decarboxylase).

The Biological Function of Photorespiration Is Unknown

Although the *PCO* oxidative photosynthetic carbon cycle recovers 75% of the carbon originally lost from the Calvin cycle as 2-phosphoglycolate, why does 2-phosphoglycolate form at all? One possible explanation is that the formation of 2-phosphoglycolate is a consequence of the chemistry of the carboxylation reaction, which requires an intermediate that can react with both CO_2 and O_2.

Such a reaction would have had little consequence in early evolutionary times if the ratio of CO_2 to O_2 in air were higher than it is today. However, the low CO_2: O_2 ratios prevalent in modern times are conducive to photorespiration, with no other function than the recovery of some of the carbon present in 2-phosphoglycolate.

Another possible explanation is that photorespiration is important, especially under conditions of high light intensity and low intercellular CO_2 concentration (e.g., when stomata are closed because of water stress), to dissipate excess ATP and reducing power from the light reactions, thus preventing damage to the photosynthetic apparatus. *Arabidopsis* mutants that are unable to photo respire grow normally under 2% CO_2, but they die rapidly if transferred to normal air. There is evidence from work with transgenic plants that photorespiration protects C_3 plants from photooxidation and photoinhibition (Kozaki and Iakeba 1996). Further work is needed to improve our understanding of the function of photorespiration.

In August 3, 2004 issue of *Proceedings of the National Academy of Sciences* of the United States of America, Shimon Rachmilevitch, Asaph B. Cousins, and Arnold J. Bloom reported that **Nitrate assimilation in plant shoots depends on photorespiration**.

They used two independent methods to show that exposure of *Arabidopsis* and wheat shoots to conditions that inhibited photorespiration also strongly inhibited nitrate assimilation. Thus, nitrate assimilation in both dicotyledonous and monocotyledonous species depends on photorespiration. This previously undescribed role for photorespiration (*i*) explains several responses of plants to rising carbon dioxide concentrations, including the inability of many plants to sustain rapid growth under elevated levels of carbon dioxide; and (*ii*) raises concerns about genetic manipulations to diminish photorespiration in crops.

As Rachmilevitch *et al* reported in their paper:

"RUBISCO catalyzes the reaction of ribulose-1,5-bisphosphate with either CO_2 or O_2 and thereby initiates, respectively, the CO_2 assimilatory (C_3 reductive) or photorespiratory (C_2 oxidative) pathways. The balance between the two reactions depends on the relative concentrations of CO_2 and O_2 at the site of catalysis. At current atmospheric levels of CO_2 (≈360 μmol mol^{-1}) and O_2 (≈209,700 μmol mol^{-1}), photorespiration in C_3 plants dissipates >25% of the carbon fixed during CO_2 assimilation. Thus, photorespiration has been viewed as a wasteful process, a vestige of the high CO_2 atmospheres under which plants evolved. At best, according to current thought, photorespiration may mitigate photoinhibition under high light and drought stress or may generate amino acids such as glycine for other metabolic pathways. Genetic modification of Rubisco to minimize photorespiration in crop plants has been the goal of many investigations.

Atmospheric CO_2 concentrations will rise to somewhere between 600 and 1,000 μmol mol^{-1} by the end of the 21st century. Transferring C_3 plants from ambient (≈360 μmol mol^{-1}) to

elevated (≈720 μmol·mol^{-1}) CO_2 concentrations decreases photorespiration and initially stimulates net CO_2 assimilation and growth by ≈30%. With longer exposures to elevated CO_2 concentrations (days to weeks), however, net CO_2 assimilation and plant growth slow down until they stabilize at rates that average 12% and 8%, respectively, above those of plants kept at ambient CO_2 concentrations. This phenomenon, known as CO_2 acclimation, is often associated with diminished activities of RUBISCO and other enzymes in the C_3 reductive photosynthetic carbon cycle. The most alarming fact is that CO_2 acclimation follows a 14% decline in overall shoot nitrogen concentrations that severely affects the plant growth."

This finding raises a serious question on the issue of food security in the years to come as due to increased fossil fuel utilization across the globe, atmospheric CO_2 concentrations will rise to somewhere between 600 and 1,000 μmol·mol^{-1} by the end of the 21st century.

C4 & CAM Pathways

THE C4 PATHWAY

The C_4 Pathway is a physiological specialisation of tropical plants that accelerates photosynthesis by concentrating Carbon Dioxide. Plants which use C4 metabolism include sugarcane, maize, sorghum, *Eleusine*, *Amaranthus*, and Switchgrass (*Panicum virgatum*). C4 plants arose during the Cenozoic Era and did not become common until the Miocene Period. Today they represent about 5% of Earth's plant biomass.

Need

Catalytic Imperfection: RUBISCO Also Catalyzes a Wasteful Oxygenase Reaction under hot conditions because of the following reasons.

The rate of the carboxylase reaction is four times that of the oxygenase reaction under normal atmospheric conditions at 25°C; the stromal concentration of CO_2 is then 10 µM and that of O_2 is 250 μM. However, when CO_2 levels are low (for example when plants in hot climates close the stomata in their leaves to conserve water but this leads to a drop in the CO_2) RuBisCO favours oxygenation of RuBP.

As temperature rises, the oxygenase activity of RUBISCO (using O_2) increases more rapidly than the carboxylase activity (using CO_2).

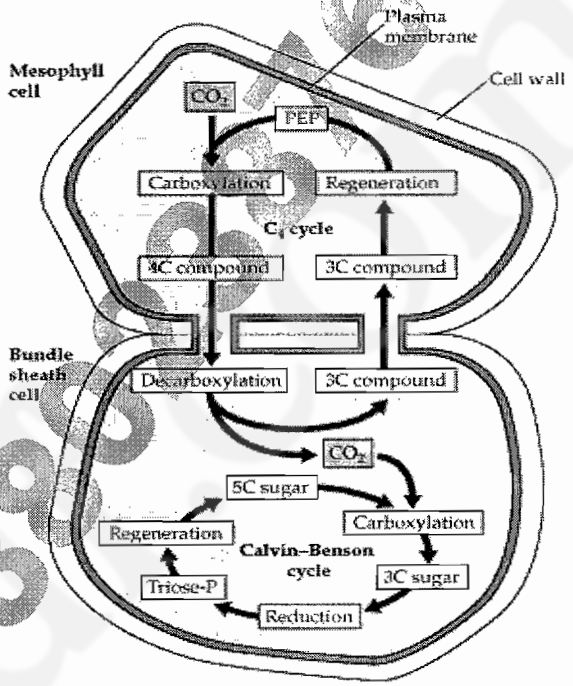
Moreover, at high temperature, the dissolubility of CO_2 in water falls sharply, hence the physiological concentration of CO_2 drops.

The products of this oxygenation of RuBP reaction are *phosphoglycolate* and *3-phosphoglycerate*. The phosphoglycolate can be salvaged and used for biosynthetic reactions but the pathway for achieving this releases CO_2 and NH_4^+ and wastes metabolic energy. Because the net result of this process is to consume O_2 and release CO_2, it is known as photorespiration.

The adaptation

The solution to this problem is to achieve a high local concentration of CO_2 at the site of the Calvin cycle in their photosynthetic cells. The essence of this process, which was elucidated by **M. D. Hatch and C. R. Slack**, is that *four-carbon (C_4) compounds such as oxaloacetate and malate carry CO_2 from mesophyll cells, which are in contact with air, to bundle-sheath cells, which are the major sites of photosynthesis*. Some plants adapted to live in hot climates, such as maize and sugar cane, have evolved a mechanism to maximize the carboxylase activity of RUBISCO. In these plants, carbon fixation using the Calvin Cycle takes place only in bundle-sheath cells that are protected from the air by mesophyll cells. Since the bundle-sheath cells are not exposed to air, the O_2 concentration is low. The CO_2 is transported from the air via the

mesophyll cells to the bundle-sheath cells by combining with three-carbon molecules (C_3) to produce four-carbon molecules (C_4). These enter the bundle-sheath cells where they are broken down to C_3 compounds, releasing CO_2.The C_3 molecules return to the mesophyll cell to accept more CO_2. This cycle ensures a high CO_2 concentration for the carboxylase activity of RUBISCO action in the bundle-sheath cells. Decarboxylation of the four-carbon compound in a bundle-sheath cell maintains a high concentration of CO_2 at the site of the Calvin cycle. The three-carbon compound pyruvate returns to the mesophyll cell for another round of carboxylation. **Since it relies on CO_2 transport via four-carbon molecules, it is called the C4 pathway and plants that use this mechanism are called C4 plants.**



The broad steps are:

1. The *C_4 pathway* for the transport of CO_2 starts in a mesophyll cell with the condensation of CO_2 and phosphoenolpyruvate to form *oxaloacetate*, in a reaction catalyzed by *phosphoenolpyruvate carboxylase.*
2. In some species, oxaloacetate is converted into *malate* by an $NADP^+$-linked malate dehydrogenase.
3. Malate goes into the bundle-sheath cell and is oxidatively decarboxylated within the chloroplasts by an $NADP^+$— linked malate dehydrogenase (or a substitute of the above enzyme).
4. The CO_2 concentration by this process can be 20-fold as great in the bundle-sheath cells as in the mesophyll cells. The released CO_2 enters the Calvin cycle in the usual way by condensing with ribulose 1,5-bisphosphate.
5. Pyruvate formed in this decarboxylation reaction returns to the mesophyll cell. Finally, phosphoenolpyruvate is formed from pyruvate by *pyruvate-P_i dikinase.*
6. The net reaction of this C_4 pathway is

$$CO_2 \text{ (in mesophyll cell)} + ATP + H_2O \longrightarrow CO_2 \text{ (in bundle-sheath cell)} + AMP + 2\,P_i + H^+$$

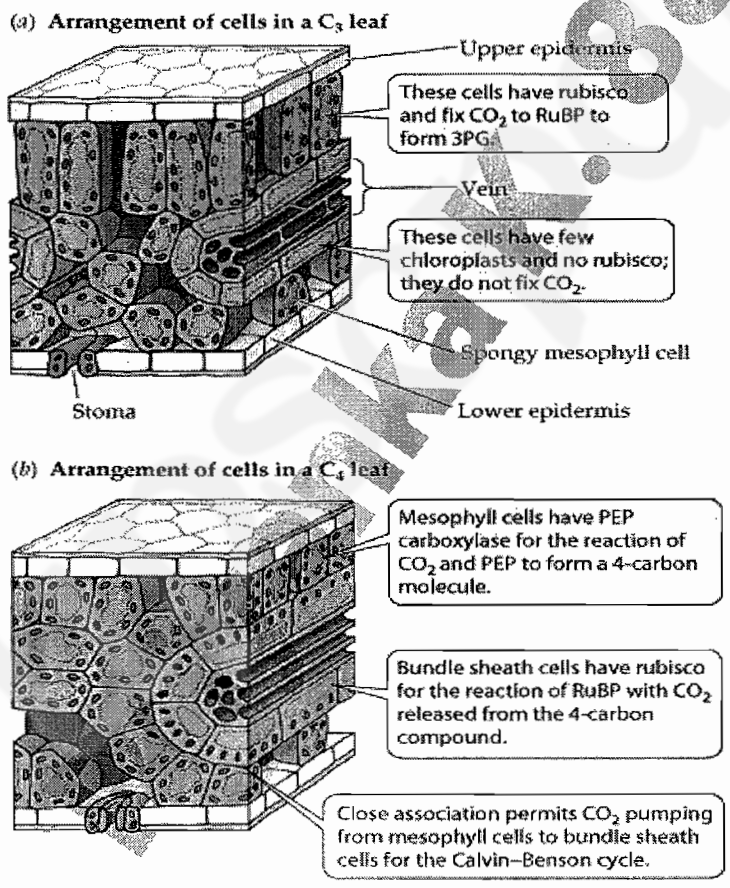
Thus, *the energetic equivalent of two ATP molecules is consumed in transporting CO_2 to the chloroplasts of the bundle-sheath cells.*

The Anatomical Specialization

Most but not all plants with C4 metabolism possess a specialized leaf anatomy called **Kranz Anatomy** (first reported by Haberlandt in 1901 as Kranz Syndrome). Over 8000 species of angiosperms, scattered among 19 different families, have developed adaptations which minimize the losses to photorespiration. The most important of the C4 metabolising families include **Aizoaceae**, **Amaranthaceae**, **Compositae**, **Chenopodiaceae**, **Cyperceae**, **Euphorbiaceae**, **Poaceae**, **Nyctaginaceae**, **Portulacaceae**, and **Zygophyllacae**.

Kranz, the German word for "halo," or "wreath,"refers to a ring of mesophyll cells just to the outside of another ring of large bundle-sheath cells, both of which encircle the vascular bundle. In transverse sections viewed under the microscope, the two cell layers give the appearance of a wreath surrounding each bundle. The rings make the Bundle Sheath, contain starch-rich chloroplasts **lacking grana** which differ from those in mesophyll cells present as the outer ring. Hence, the chloroplasts are called dimorphic. This peculiar anatomy is called Kranz Anatomy.

In addition to the unique "wreaths," other features that typify leaves with Kranz anatomy include small intercellular spaces, and frequent veins.



Kranz anatomy and the C_4 photosynthetic pathway are especially characteristic of tropical grasses such as sugar cane, where it was first discovered, and corn, although it has also been found in other plants.

A comparison of Kranz Anatomy with normal C3 leaf anatomy is given below.

Is Kranz Anatomy Universal in C4 Plants?

The Answer is No. We have three well known examples to support this.

On 26 October 2000, Nature reported the discovery of both the C_3 and C_4 pathways in a marine diatom *Thallassiosira*. In this unicellular organism, the two paths are kept separate by having

the C_4 path in the cytosol, and the C_3 path confined to the chloroplast. The presence of a C_4 pathway probably reflects the frequent low concentrations of CO_2 in ocean waters.

A report in the 24 January 2002 issue of Nature (by Julian M. Hibbard and W. Paul Quick) describes the discovery that tobacco, a C_3 plant, has cells capable of fixing carbon dioxide by the C_4 path. These cells are clustered around the veins (containing xylem and phloem) of the stems and also in the petioles of the leaves. In this location, they are far removed from the stomata that could provide atmospheric CO_2. Instead, they get their CO_2 and/or the 4-carbon malic acid in the sap that has been brought up in the xylem from the roots.

In 2003, Voznesenskaya, Elena V and co-workers showed that C_4 photosynthesis can function within a single photosynthetic cell in terrestrial plants. *Borszczowia aralocaspica* (now known as *Sueda aralocapsica*) and *Bienertia cycloptera* (Chenopodiaceae) lack Kranz anatomy and have carbon isotope values like C_4 and CAM plants. Lack of nighttime CO_2 fixation indicates they are not CAM. Their photosynthetic responses to varying CO_2 and O_2 are typical of C_4 plants with Kranz anatomy. These species exhibit independent, novel solutions for the function of the C_4 mechanism through spatial compartmentation of dimorphic chloroplasts and other organelles, and photosynthetic enzymes, in distinct positions within a single chlorenchyma cell. Two inter-connected chloroplast-containing cytoplasmic compartments in the chlorenchyma cells are spatially separated.

Benefits of C4 Metabolism

Tropical plants with a C_4 pathway do little photorespiration because the high concentration of CO_2 in their bundle-sheath cells accelerates the carboxylase reaction relative to the oxygenase reaction. This effect is especially important at higher temperatures. The geographic distribution of plants having this pathway (C_4 plants) and those lacking it (C_3 plants) can now be understood in molecular terms. C_4 plants have the advantage in a hot environment and under high illumination, which accounts for their prevalence in the tropics. C_3 plants, which consume only 18 molecules of ATP per hexose molecule formed in the absence of photorespiration (compared with 30 molecules of ATP for C_4 plants), are more efficient at temperatures of less than about 28°C, and so they predominate in temperate environments.

C4 pathway also allows water economy, because the stomata can open for relatively shorter periods in the presence of CO_2 concentration mechanisms.

Evolution of the C4 Pathway

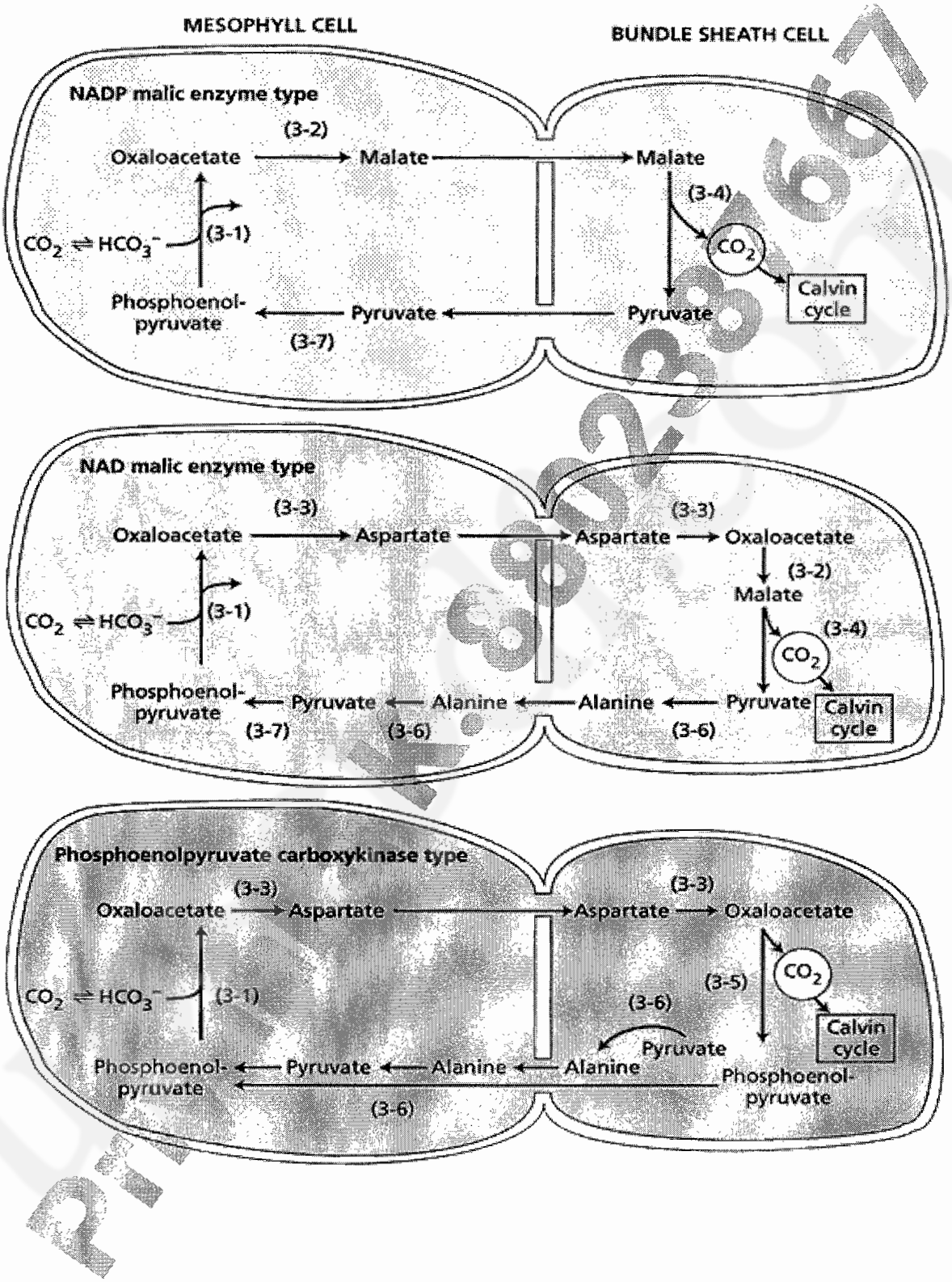
RUBISCO is found in bacteria, eukaryotes, and even archaea, though other photosynthetic components have not been found in archaea. Thus, RUBISCO emerged early in evolution, when the atmosphere was rich in CO_2 and almost devoid of O_2. The enzyme was not originally selected to operate in an environment like the present one, which is almost devoid of CO_2 and rich in O_2. Photorespiration became significant about 60 million years ago, when the CO_2 concentration fell to present levels. The C_4 pathway is thought to have evolved in response to this selective pressure. It is interesting to note that none of the enzymes are unique to C_4 plants, suggesting that this pathway was created using existing enzymes.

C4 photosynthesis is an evolutionary solution to high rates of photorespiration and low photosynthetic efficiency caused by high temperature and low atmospheric CO_2. The C4 pathway evolved independently over 45 times in 19 families of angiosperms, and thus represents one of the most convergent of evolutionary phenomena. C4 photosynthesis is restricted to advanced angiosperm taxa that largely diversified in the past 40 million years, indicating the rise of advanced angiosperms is an important precondition for C4 evolution. C4 photosynthesis probably first arose in grasses during the Oligocene epoch. The earliest C4 dicots are Chenopodiaceae species present 15 to 21 million years ago; however, most C4 dicot lineages are estimated to have appeared in the past 5 million years. Recent C4 lineages provide insights into the environmental imperatives favoring C4 evolution, because the C4 ancestors and C3-C4 intermediate species still exist. C4 photosynthesis in recent dicot lineages originated in arid regions of low latitude, implicating combined effects of heat, drought and/or salinity as important conditions promoting the origin of C4 plants. Low atmospheric CO_2 is a significant contributing factor for C4 evolution, because it is required for high rates of photorespiration. Consistently, the diversification of C4 plants in the evolutionary record coincides with a periods of increasing global aridification and declining atmospheric CO_2.

The Variants of the C4 Pathway

Please refer to the diagram on the next page.

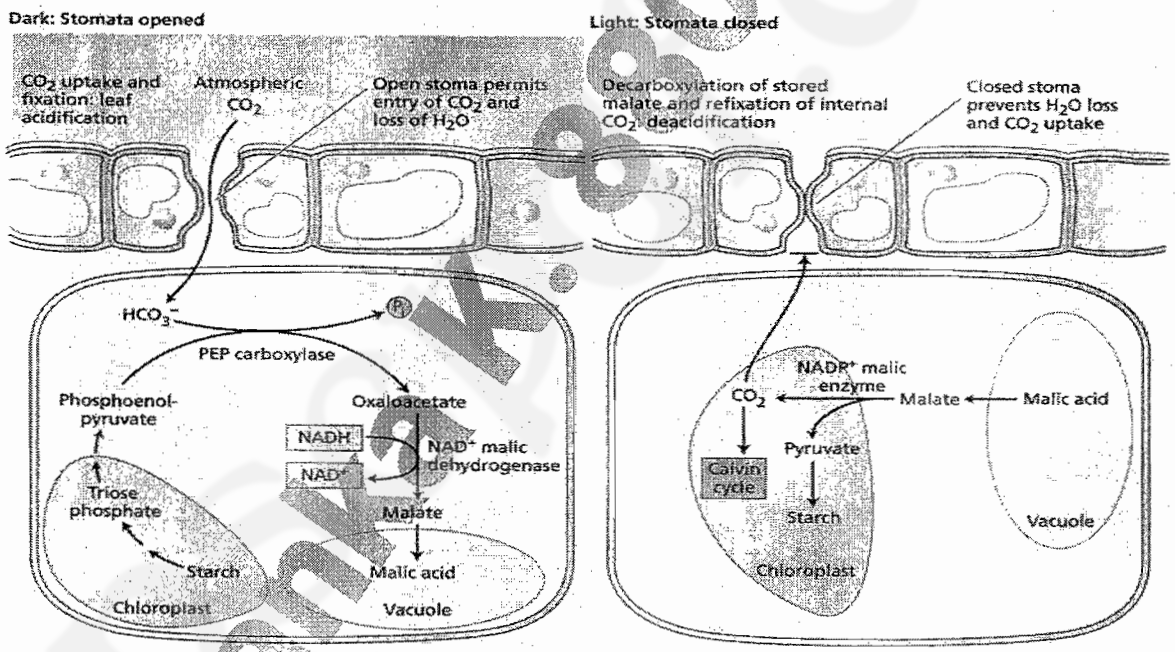
There are three variants of the C_4 photosynthetic carbon cycle. The variants differ principally in (1) the nature of the four-carbon acid (malate or aspartate) transported into the bundle sheath cell and of the three-carbon acid (pyruvate or alanine) returned to the mesophyll cell and (2) the nature of the enzyme that catalyzes the decarboxylation step in the bundle sheath cell. The three variants are named after the enzymes that catalyze the decarboxylation reactions. Representatives of each variant include maize, crabgrass, sugarcane, sorghum (NADP malic enzyme); pigweed, millet (NAD malic enzyme); guinea grass (phosphoenolpyruvate carboxykinase).



CAM PATHWAY

Crassulacean acid metabolism (CAM) is a carbon fixation pathway in some photosynthetic plants. CAM is usually found in plants living under arid conditions, including those found in the desert (for example, cacti or pineapple). It is named after the plant family it was first discovered in, the Crassulaceae.

Crassulacean acid metabolism (CAM) is yet another adaptation to increase the efficiency of the Calvin cycle. Crassulacean acid metabolism, named after the genus *Crassulacea* (the succulents), is a response to drought as well as warm conditions. In CAM plants, the stomata of the leaves are closed in the heat of the day to prevent water loss. As a consequence, CO_2 cannot be absorbed during the daylight hours when it is needed for glucose synthesis. When the stomata open at the cooler temperatures of night, CO_2 is fixed by the C_4 pathway into malate, which is stored in vacuoles. During the day, malate is decarboxylated and the CO_2 becomes available to the Calvin cycle. In contrast with C_4 plants, CO_2 accumulation is separated from CO_2 utilization temporally in CAM plants rather than spatially.



evolution
A Centre of Excellence for Civil Service Examination Guidance
B 11, Second Floor, Commercial Complex, Batra Cinema Road
Next to ICICI A.T.M., Dr. Mukherjee Nagar, DELHI - 110 009
Phone: 011 - 32974645, 32974651, 47092329

Part – III: Lipid metabolism

evolution
Reference Study Material

Lipid metabolism in plants

An introduction to lipids

Lipids are hydrophobic molecules that function as energy storage molecules, signaling molecules, and the major components of cell membranes.

Lipids are defined by their physicochemical properties. This makes them different from all of the other biomolecules. Rest other biomolecule types are defined by their functional groups.

The lipids have the following properties in common.

- No net charge
- Non-polarity in the pure form (Some conjugated lipids such as phospholipids may have a polar part)
- No solubility in water
- Solubility in organic solvents such as alcohol, ether, acetone etc.

Lipids include fats and oils, waxes, phospholipids, steroids (like cholesterol), and some other related compounds.

Lipids have three major roles in cells.

1. They provide an important form of energy storage.
2. Lipids are the major components of cell membranes.
3. Lipids play important roles in cell signaling, both as steroid hormones (e.g., estrogen and testosterone in animals and *Brassinosteroids* in plants) and as second messenger molecules within the cell.

Important types of lipids

Fatty acids, triacylglyrols, phospholipids and glycolipids

The simplest lipids are fatty acids, which consist of long hydrocarbon chains, most frequently containing 16 or 18 carbon atoms, with a carboxyl group (COO-) at one end.

There are two types of fatty acids:

1. **Unsaturated fatty acids** contain one or more double bonds between carbon atoms.
2. **Saturated fatty acids** have all of the carbon atoms bonded to the maximum number of hydrogen atoms.

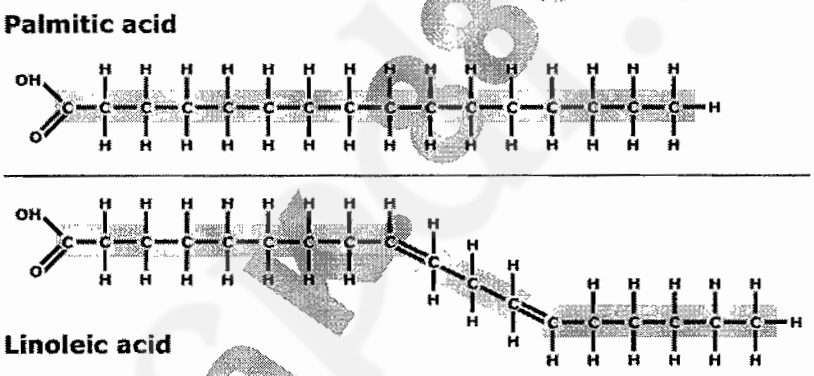


Figure: Saturated and unsaturated fatty acid

The long hydrocarbon chains of fatty acids contain only nonpolar C—H bonds, which are unable to interact with water. The hydrophobic nature of these fatty acid chains is responsible for much of the behavior of complex lipids.

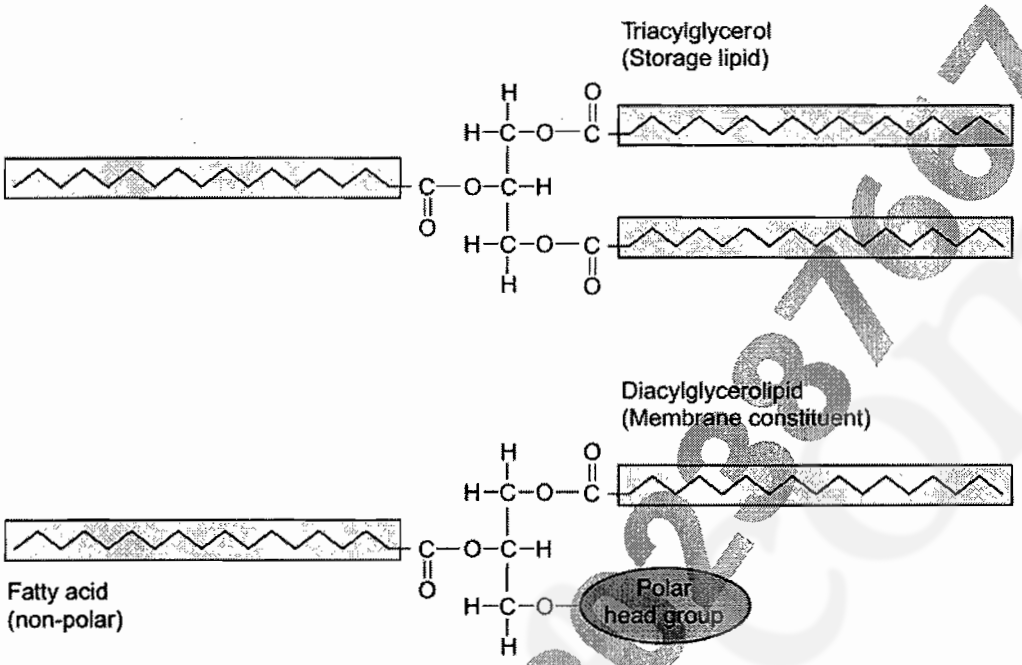


Figure: Triacylglycerol and Diacylglycerolipid

Fatty acids are stored in two main forms.

1. **Triacylglycerols** (**triglycerides**), or fats, which consist of three fatty acids linked to a glycerol molecule. Triacylglycerols are insoluble in water and therefore accumulate as fat droplets in the cytoplasm. When required, they can be broken down for use in energy-yielding reactions. It is noteworthy that fats are a more efficient form of energy storage than carbohydrates, yielding more than twice amount of energy per weight of material broken down.
2. **Diacylglycerolipid**, the principal components of cell membranes, consist of two fatty acids joined to a polar head group. In the **glycerophospholipids**, the two fatty acids are bound to carbon atoms in glycerol, as in triacylglycerols. The third carbon of glycerol, however, is bound to a phosphate group, which is in turn frequently attached to another small polar molecule, such as choline, serine, inositol, or ethanolamine.

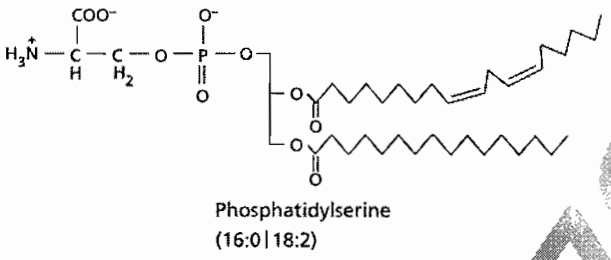


Figure: A Glycerophospholipid

When the polar group of the diacylglycerolipid is a carbohydrate, it is known as **glyceroglycolipid**.

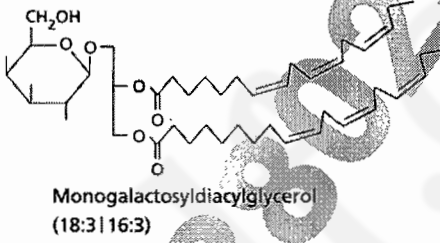


Figure: A glyceroglycolipid

All phospholipids have hydrophobic tails, consisting of the two hydrocarbon chains, and hydrophilic head groups, consisting of the phosphate group and its polar attachments. Consequently, phospholipids are **amphipathic molecules**, partly water-soluble and partly water-insoluble. This property of phospholipids is the basis for the formation of biological membranes.

Sterols

Sterols are a subgroup of the steroids. They occur naturally in plants, animals, and fungi, with the most familiar type of animal sterol being cholesterol. Cholesterol is vital to animal cell membrane structure and function as a precursor to fat-soluble vitamins and steroid hormones.

Sterols consist of four hydrocarbon rings. The hydrocarbon rings are strongly hydrophobic, but the hydroxyl (OH) group attached to one end of cholesterol is weakly hydrophilic, so cholesterol is also amphipathic.

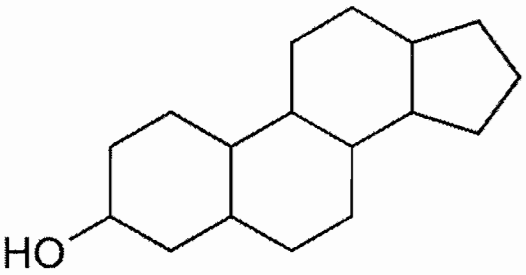


Figure: Sterol general structure

Lipid synthesis in plants

Fatty acid biosynthesis involves the cyclic condensation of two-carbon units in which acetyl-CoA is the precursor. In plants, fatty acids are synthesized exclusively in the plastids; in animals, fatty acids are synthesized primarily in the cytosol.

The enzymes of the pathway are thought to be held together in a complex that is collectively referred to as fatty acid synthase. The complex probably allows the series of reactions to occur more efficiently than it would if the enzymes were physically separated from each other. In addition, the growing acyl chains are covalently bound to a low-molecular-weight, acidic protein called acyl carrier protein (ACP). When conjugated to the acyl carrier protein, the fatty acid chain is referred to as acyl-ACP.

The steps are as follows.

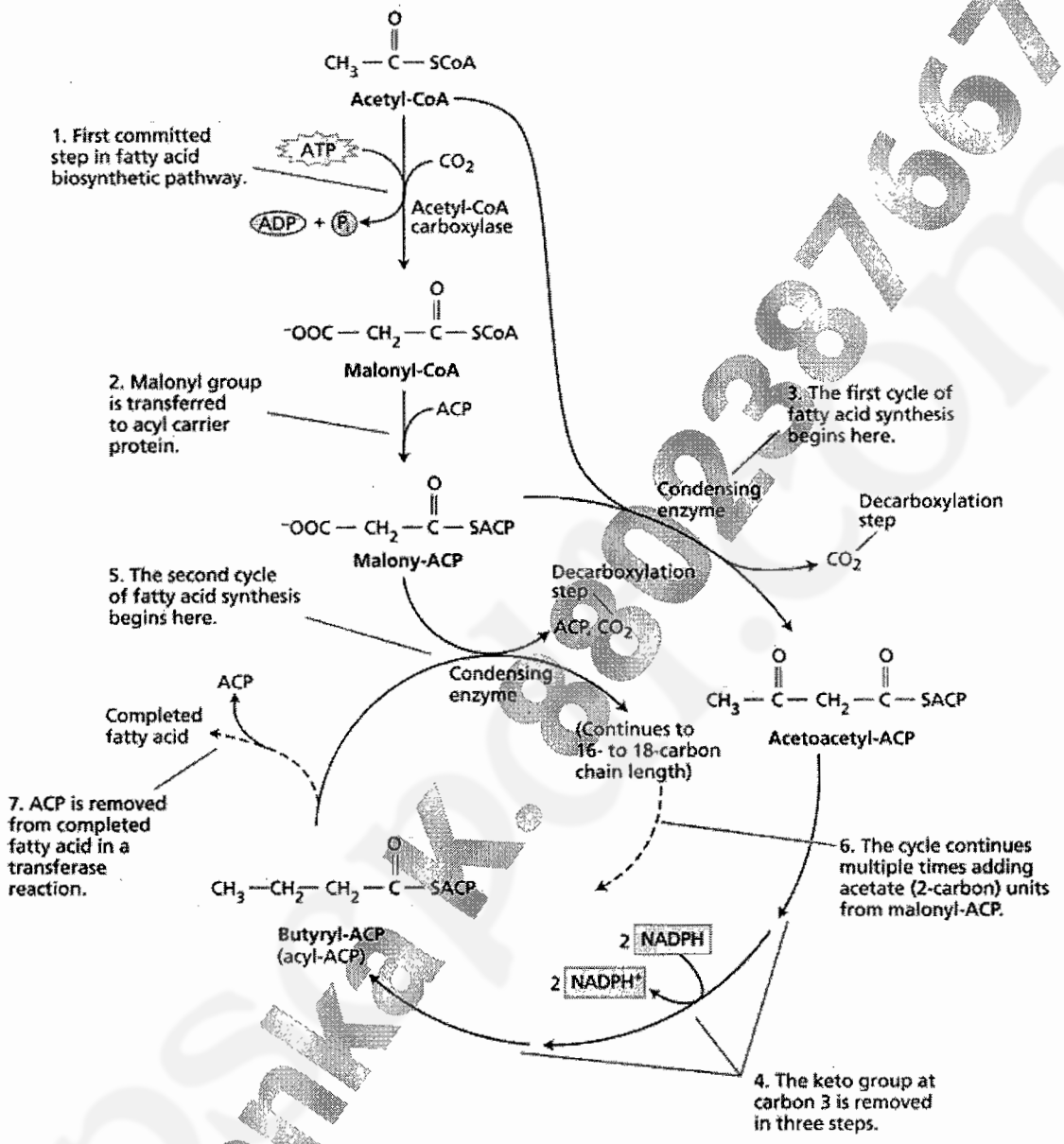


Figure: Fatty acid biosynthesis

The fatty acids synthesized in the plastid are next used to make the glycerolipids of membranes and oleosomes. The first steps of glycerolipid synthesis are two acylation reactionswhich transfer fatty acids from acyl-ACP or acyl-CoA to glycerol-3-phosphate to form phosphatidic acid. The action of a specific phosphatase produces diacyl-glycerol (DAG) from phosphatidic acid.

Lipid breakdown

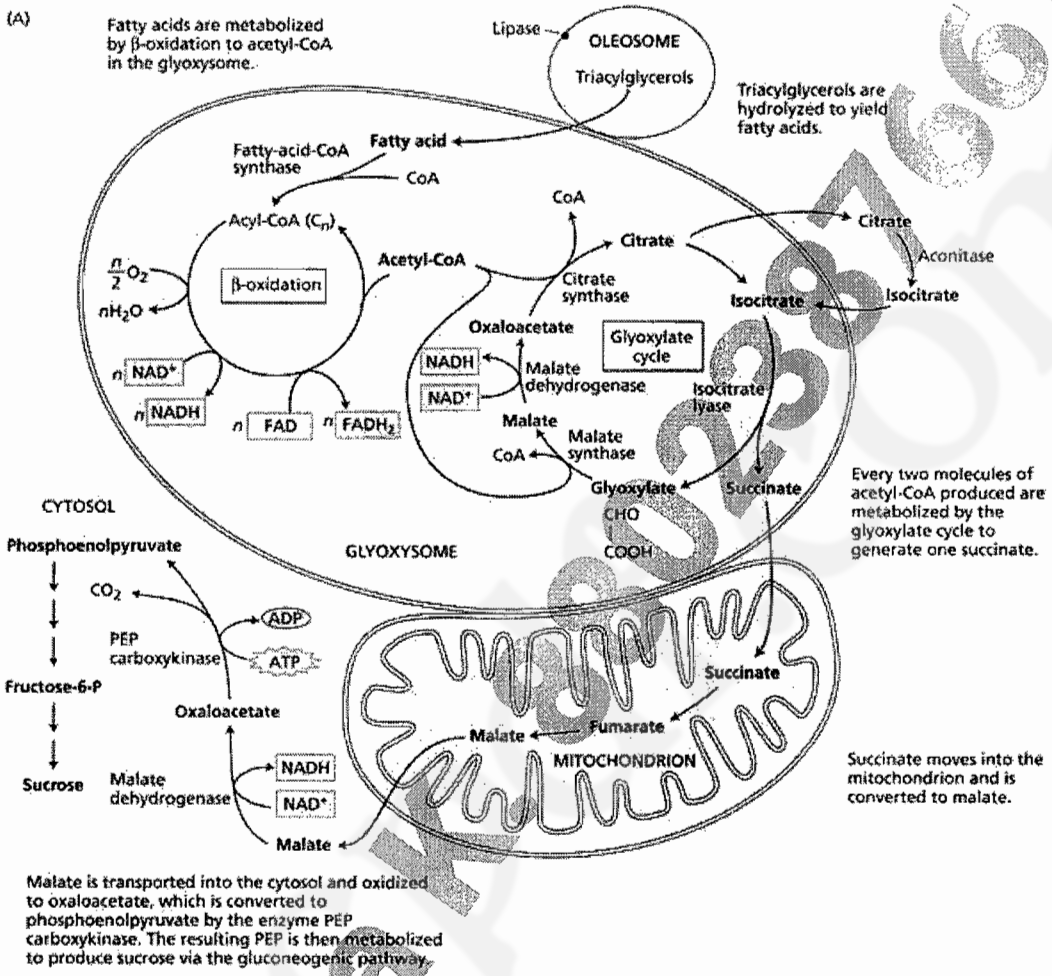


Figure: Lipid breakdown and generation of sugars

Lipase hydrolysis

The initial step is the breakdown of triglycerides stored in the oil bodies by the enzyme lipase, which is located on the half-membrane that serves as the outer boundary of the oil body. The lipase hydrolyzes triacylglycerols to three molecules of fatty acid and glycerol. Corn and cotton also contain a lipase activity in the oil body, but peanut, soybean, and cucumber show lipase activity in the glyoxysome instead. During the breakdown of lipids, oil bodies and glyoxysomes are generally in close physical association.

Beta-oxidation

After hydrolysis of the triacylglycerols, the resulting fatty acids enter the glyoxysome, where they are activated by conversion to fatty-acyl-CoA by the enzyme fatty-acyl-CoA synthase. Fatty-acyl-CoA is the initial substrate for the ß-oxidation series of reactions, in which Cn fatty acids (fatty acids composed of n number of carbons) are sequentially broken down to n/2 molecules of acetyl-CoA. This reaction sequence involves the reduction of 1⁄2 O2 to H2O and the formation of 1 NADH and 1 FADH2 for each acetyl-CoA produced.

In mammalian tissues, the four enzymes associated with ß-oxidation are present in the mitochondrion; in plant seed storage tissues, they are localized exclusively in the glyoxysome. Interestingly, in plant vegetative tissues (e.g., mung bean hypocotyl and potato tuber), the ß-oxidation reactions are localized in a related organelle, the peroxisome.

The glyoxylate cycle

The function of the glyoxylate cycle is to convert two molecules of acetyl-CoA to succinate. The acetyl-CoA produced by ß-oxidation is further metabolized in the glyoxysome through a series of reactions that make up the glyoxylate cycle. nitially, the acetyl-CoA reacts with oxaloacetate to give citrate, which is then transferred to the cytoplasm for isomerization to isocitrate by aconitase. Isocitrate is reimported into the peroxisome and converted to malate by two reactions that are unique to the glyoxylate pathway.

1. First isocitrate (C6) is cleaved by the enzyme isocitrate lyase to give succinate (C4) and glyoxylate (C2). This succinate is exported to the motochondria.
2. Next malate synthase combines a second molecule of acetyl-CoA with glyoxylate to produce malate.

Malate is then oxidized by malate dehydrogenase to oxaloacetate, which can combine with another acetyl-CoA to continue the cycle. The glyoxylate produced

keeps the cycle operating in the glyoxysome, but the succinate is exported to the mitochondria for further processing.

The mitochondrial role. Moving from the glyoxysomes to the mitochondria, the succinate is converted to malate by the normal citric acid cycle reactions. The resulting malate can be exported from the mitochondria in exchange for succinate via the dicarboxylate transporter located in the inner mitochondrial membrane. Malate is then oxidized to oxaloacetate by malate dehydrogenase in the cytosol, and the resulting oxaloacetate is converted to carbohydrate.